VOLUME XXXI JUNE 1939 No. 2

ON THE AFRICAN STREBLIDAE (DIPTERA ACALYPTERAE) INCLUDING THE MORPHOLOGY OF THE GENUS *ASCODIPTERON* ADENS. AND A DESCRIPTION OF A NEW SPECIES

BY B. JOBLING, F.R.E.S.

Wellcome Entomological Field Laboratories, Wellcome Bureau of Scientific Research, London

(With 4 Figures in the Text)

CONTENTS

	PAGE
Introduction	147
Subfamily Nycteriboscinae, genus *Nycteribosca* Speiser, 1900	149
Key to the African species of the genus *Nycteribosca*	150
Nycteribosca africana Walker	150
Nycteribosca alluaudi Falcoz	152
Nycteribosca bequaerti Jobling	152
Genus *Raymondia* Frauenfeld, 1855	152
Key to the African species of the genus *Raymondia*	152
Raymondia huberi Frauenfeld	154
Raymondia setosa Jobling	154
Raymondia planiceps Jobling	154
Raymondia alulata Speiser	155
Raymondia waterstoni Jobling	155
Raymondia intermedia Jobling	155
General morphology of the genus *Ascodipteron*	155
The life history and the structure of the larva of *Ascodipteron africanum* sp.n.	161
The systematic position and the definition of the genus *Ascodipteron*	162
Ascodipteron africanum sp.n.	162
Ascodipteron lophotes Monticelli	163
Ascodipteron tabulatum Speiser	163
Appendix, amendment of name	164
References	164

INTRODUCTION

DR F. W. EDWARDS has kindly submitted to me the specimens of Streblidae collected by him in March 1935, during his entomological expedition to East Africa, in the caves near Kapretwa, at 6500 ft., on Mt Elgon, Kenya. To this

material he added the specimens collected by Dr G. H. E. Hopkins, in the same locality, and asked me to write a short report.

After an examination of these two collections, I came to the conclusion that a short report on these specimens will have very little value. Therefore, I have decided to write this paper, which includes all the records of the African Streblidae known to me at present, and an illustrated key for their identification.

Amongst the material of Dr Hopkins there were thirteen spirit specimens of bats infested with the endoparasitic females of the genus *Ascodipteron*. With this material I was able to study the external structure of this endoparasite, and the description of it is given in this paper. I think that this description will serve as a basis for the further study of the characters of the species of this highly specialized genus.

As I could not refer the specimens of *Ascodipteron* to either *A. lophotes* Monticelli (1898), or to *A. tabulatum* Speiser (1908*a*), they are described here as a new species. However, I must mention that the descriptions of these authors are incomplete, and a comparison of my type specimen with theirs may demonstrate its identity with either *A. lophotes* or *A. tabulatum*.

The African species of this family of Diptera belong to the following three genera: *Raymondia*, *Nycteribosca* and *Ascodipteron*. The first two genera are included in the subfamily Nycteriboscinae and the last in the Ascodipterinae. The species of these genera are widely distributed in the Oriental region, where those of the genus *Nycteribosca* are more numerous than in Africa. But none of the Oriental species of these genera occur in Africa and vice versa. Three Ethiopian species, *Nycteribosca africana* Walker, *N. alluaudi* Falcoz and *Raymondia huberi* Frauenfeld, are common on certain bats in the Palaearctic region of Africa. Of these species *Nycteribosca africana* has the widest distribution, and is the only streblid which occurs in the Mediterranean parts of Europe and Asia (Portugal, south of France, Sardinia, Cyprus and Palestine).

Table I shows that the African Streblidae parasitize bats belonging to eight families of Chiroptera. The more common and widely distributed species, such as *N. africana* and *Raymondia huberi*, have been found on the species of the three families of bats, whereas the less common species, with the more localized distribution, have been recorded only from one, or from two species of bats of the same genus.

It seems that the evolution of the Streblidae has been going on side by side with that of their hosts. The most generalized species of this family occur on the species of the most generalized family of Chiroptera. Thus *Nycteribosca bequaerti* and the Oriental species, *N. gigantea*, which are the most generalized species of the Streblidae, have been found only on bats of the most primitive form of Pteropidae of the suborder Megachiroptera.

The distribution of the African Streblidae is known very incompletely, for instance, there is only one record of these Diptera from the whole of West Africa (Old Calabar, South Nigeria), none from French Equatorial Africa and only two from the Belgian Congo.

Table I. *Showing the families, genera and species of bats on which the species of African Streblidae have been found*

	Genera and species of Streblidae											
	Nycteribosca			*Raymondia*						*Ascodipteron*		
	N. africana	*N. alluaudi*	*N. bequaerti*	*R. huberi*	*R. setosa*	*R. planiceps*	*R. alulata*	*R. waterstoni*	*R. intermedia*	*A. lopholes*	*A. tabulatum*	*A. africanum*
PTEROPIDAE:												
Rousettus leachi Smith	.	.	+	.	.	.	.	.	.	.	.	.
RHINOPOMIDAE:												
Rhinopoma microphyllum Geoffroy	+	.	.	.	.	.	.	.	.	.	.	.
EMBALLONURIDAE:												
Taphozous hildegardeae Thomas	.	+	.	.	.	.	.	.	.	.	.	.
Coleura afra Peters	.	.	.	+	.	.	.	.	.	.	.	.
NYCTERIDAE:												
Nycteris damarensis Peters	.	.	.	.	.	.	+	.	.	.	.	.
N. capensis Smith	.	.	.	.	.	.	+	.	.	.	.	.
MEGADERMIDAE												
Cardioderma cor Peters	.	.	.	+	.	+	.	.	.	.	.	.
RHINOLOPHIDAE:												
Rhinolophus augur augur Andersen	+	.	.	.	.	.	.	.	.	.	.	.
R. augur geoffroyi Andersen	+	.	.	.	.	.	.	+	.	.	.	.
R. blasii Peters	+	.	.	.	.	.	.	.	.	.	.	.
R. capensis Lichtenstein	+	.	.	.	.	.	.	.	.	.	.	.
R. empusa Andersen	+	.	.	.	.	.	.	+	.	.	.	.
R. euryale Blas	+	.	.	.	.	.	.	.	.	.	.	.
R. ferrum equinum Schreber	+	.	.	.	.	.	.	.	.	.	.	.
R. hildebrandti Andersen	.	.	.	.	.	.	.	.	+	.	.	.
R. hipposideros Bechstein	+	.	.	.	.	.	.	.	.	.	.	.
R. lobatus Peters	.	.	.	.	.	.	.	.	+	.	.	.
R. meheleyi Matschie	+	.	.	.	.	.	.	.	.	.	.	.
R. simulator Andersen	.	.	.	.	.	.	.	+	.	.	.	.
R. clivosus Cretzschmar	.	.	.	.	.	.	.	.	.	+	.	.
HIPPOSIDERIDAE:												
Cloeotis percivali australis Roberts	.	.	.	+	.	.	.	+	.	.	.	.
Triaenops persicus Dobson	.	.	.	+	.	.	.	.	.	.	.	.
Hipposideros caffer Sundevall	.	.	.	+	.	.	.	.	.	.	.	.
H. fuliginosus Temminck	.	.	.	+	.	.	.	.	.	.	.	.
Asellia tridens Geoffroy	+	.	.	+	.	.	.	.	.	.	.	.
VESPERTILIONIDAE:												
Miniopterus schreibersi Kuhl	+	.	.	.	.	.	.	.	.	.	.	.
M. natalensis arenarius Heller	.	.	.	.	.	.	.	.	.	.	.	+
Miniopterus sp.	.	.	.	.	.	.	.	.	.	.	+	.
Vespertilio murinus Linn.	+	.	.	.	.	.	.	.	.	.	.	.

Subfamily NYCTERIBOSCINAE

Genus **Nycteribosca** Speiser, 1900

Head broad in its anterior part, becoming narrow towards occipital foramen, its dorsal and ventral surfaces convex; no depressions for the fore-coxae. One-faceted eyes present; postvertex (vertical triangle) distinct; arista of antennae flagelliform, with a few branches in its distal half; distal end of palps rounded. Thorax almost spherical, with very distinct, complete mesonotal suture; humeral calluses absent; scutellum with many setae usually of variable number and size. Wings more than twice as long as broad, with five longitudinal

veins; first longitudinal vein not much thicker than the second, with setae in its distal part only; numerous setae in humeral and costal cells, those near costal margin and in humeral cell distinctly longer; alula well developed and fringed with setae.

KEY TO THE AFRICAN SPECIES OF THE GENUS *NYCTERIBOSCA*

1. Head dark, i.e. distinctly more pigmented than the body; middle part of mesonotum bare; wings 4·6 mm. long (Fig. 1A) **N. bequaerti** Jobling.
   Head not darker than the body; mesonotum uniformly setose 2

2. Distal part of second longitudinal vein gently bent towards costal margin, third and fourth longitudinal veins divergent near the apex of the wing (Fig. 1B); second sternite cordiform (Fig. 1C, *st.* 2); wings 2·6–2·9 mm. long **N. africana** Walker.

   Distal part of second longitudinal vein sharply bent towards costal margin, third and fourth longitudinal veins parallel near the apex of the wing (Fig. 1D); second sternite triangular, not quite distinct (Fig. 1E, *st.* 2); wings 3 mm. long **N. alluaudi** Falcoz.

Nycteribosca africana (Walker, 1849) Speiser, 1900

Strebla africana Walker, 1849.
Raymondia kollari Frauenfeld, 1855 (*nec* Schiner, 1868).
Strebla kollari Kolenati, 1856.
Brachytarrina kollari Kolenati, 1863.
Nycteribosca kollari Speiser, 1900.

This is the commonest African species of the genus, and is the only streblid which occurs in the Mediterranean parts of the Palaearctic region. Its distribution extends throughout Africa to the Cape of Good Hope, where the type specimen was collected. According to Falcoz (1923, 1924 and 1926) it occurs in Portugal, south of France and Sardinia. He recorded it from *Rhinolophus euryale* Blas, *Asellia tridens* Geoffroy, *Rhinopoma microphyllum* Geoffroy and *Vespertilio murinus* Linn.; from *Rhinolophus hipposideros* Bechstein, *R. meheleyi* Matschie and *Miniopterus schreibersi* Kuhl, from Algeria; and from *Rhinolophus ferrum equinum* Schreber, from Morocco. There are specimens in the collection of the British Museum (Natural History), from Cintra, Portugal. The species has also been found on bats in Cyprus, Tunis and Egypt. Dr P. A. Buxton collected many specimens from *Rhinolophus blasii* Peters, in Jerusalem, Palestine. Bedford (1936) recorded it from *Rhinolophus empusa* Andersen, near Krugersdorp, Transvaal. It is apparently common in South Africa, because there are specimens in the collection of the British Museum (Natural History) from Somaliland; from *Rhinolophus capensis* Lichtenstein, Durban; from *Rhinolophus augur augur* Andersen, Kingersdorp District; and from *Rhinolophus augur geoffroyi* Andersen, collected near Pretoria. This year it has been taken by G. H. E. Hopkins, from an undetermined bat, on Mt Elgon, 6300 ft. near Kapretwa, Kenya.

The type specimen of this species is preserved in the collection of the British Museum (Natural History).

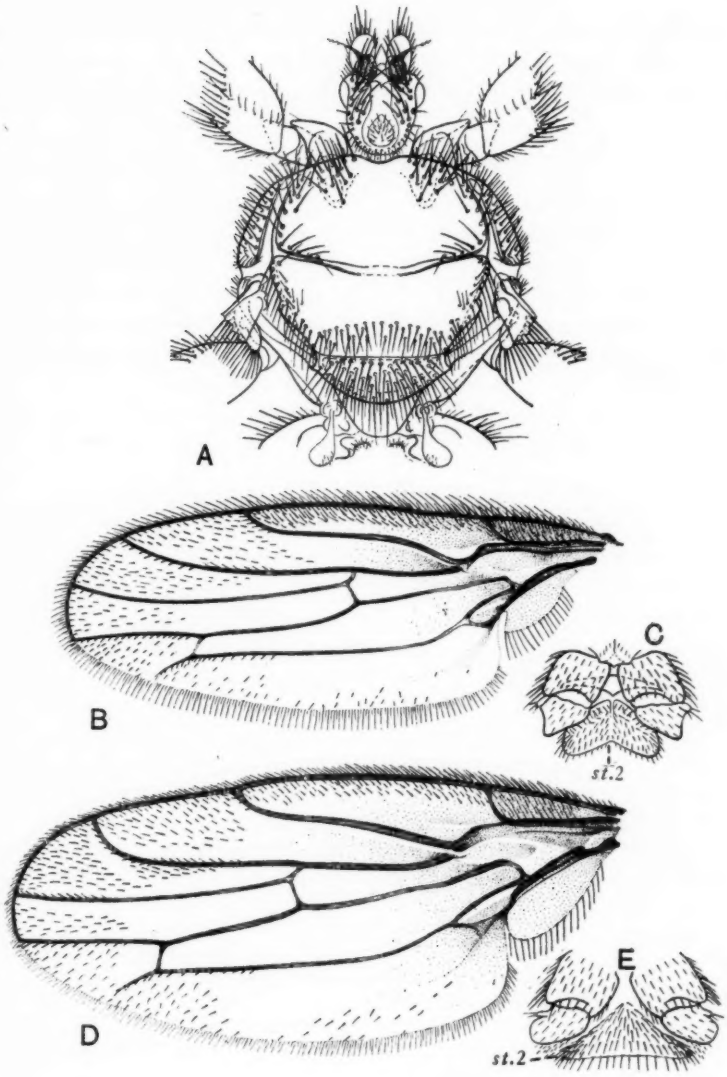

Fig. 1. A, head and thorax of *Nycteribosca bequaerti* Jobling; B, wing of *N. africana* Walker; C, ventral view of basal part of abdomen of same; D, wing of *N. alluaudi* Falcoz; E, ventral view of basal part of abdomen of same: *st.* 2, second sternite. (After Jobling, *Parasitology*, 1934 and 1936.)

Nycteribosca alluaudi Falcoz, 1923

The type specimen was recorded from *Taphozous hildegardeae* Thomas, from British East Africa, and it is in Dr L. Falcoz's collection. This species has also been recorded from Luxor and Alexandria, Egypt, both from undetermined bats.

Nycteribosca bequaerti Jobling, 1936

It is the largest African species, with darkly pigmented head. In its general appearance it resembles very much the Oriental species, *N. gigantea*, from which, however, it can be easily separated by several distinctive characters. The specimens were collected by Mr A. Loweridge, from *Rousettus leachi* Smith, Bagilo, Uluguru Mountains, Tanganyika Territory. The type and the two paratypes are in the collection of Dr J. C. Bequaert and one paratype is in the collection of the Wellcome Entomological Field Laboratory.

I am sure that the specimen which was collected in East Africa, and identified by Speiser (1908*a*) as *Nycteribosca gigantea*, belongs to this species.

Genus **Raymondia** Frauenfeld, 1855

Head rounded laterally, as broad as the anterior part of thorax, or more or less quadrangular and narrower than the thorax, dorso-ventrally flattened, closely articulated to the thorax, with concavity on each side of occipital foramen for the fore-coxae. Eyes absent; postvertex (vertical triangle) absent; arista of antennae short and thick, with many branches; palps nearly as long as broad, obtuse. Thorax somewhat flattened, mesonotal suture not well defined or incomplete in the middle; humeral calluses very distinct; scutellum always with two very strong, long setae and a few small usually marginal setae. Wings twice as long as broad, with five longitudinal veins; first longitudinal vein much thicker than the second vein, with setae along its entire length; very few setae, or none in humeral and costal cells; alula not always well developed, without, or with one or a few setae.

KEY TO THE AFRICAN SPECIES OF THE GENUS *RAYMONDIA*

1. Head subquadrangular, narrower than anterior margin of thorax; theca of labium as long as or a little longer than broad 2
   Head rounded, slightly broader than anterior margin of thorax; theca (*th.*) of labium much broader than long (Fig. 2A); wings 1·7 mm. long **R. planiceps** Jobling.
2. Alula vestigial, without setae (Fig. 2I, *a*) 5
   Alula distinct, with one or a few setae 3
3. Postero-dorsal margin of head without setae (Fig. 2D); alula with one terminal setae (Fig. 2E) 4
   Postero-dorsal margin of head with setae (Fig. 2B); alula with four or five setae (Fig. 2C); wings broad, 1·4 mm. long **R. alulata** Speiser.

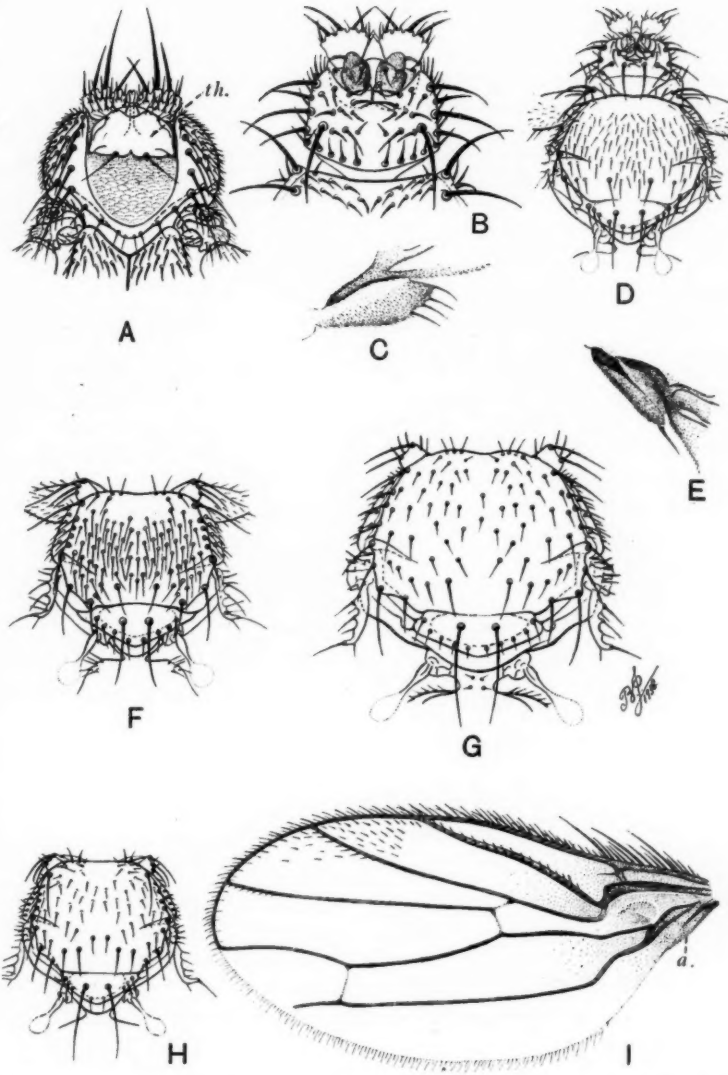

Fig. 2. A, ventral view of head and theca of labium (*th.*) of *Raymondia planiceps* Jobling; B, head of *R. alulata* Speiser; C, alula of same; D, head and thorax of *R. huberi* Frauenfeld; E, alula of same; F, thorax of *R. setosa* Jobling; G, thorax of *R. waterstoni* Jobling; H, thorax of *R. intermedia* Jobling; I, wing of same: *a*, alula. (A, B, D, F, G, H and I after Jobling, *Parasitology*, 1930, 1931 and 1936.)

4. Setae on prescutum about as long as the distance between them (Fig. 2D)
R. huberi Frauenfeld.

Setae on prescutum about twice as long as the distance between them (Fig. 2F)
R. setosa Jobling.

5. Anterior margin of prescutum rounded, with a slight incurvature in the middle; marginal setae of scutellum stand at equal intervals; third and fourth longitudinal veins parallel near the apex of the wing (Fig. 2G) **R. waterstoni** Jobling.

Anterior margin of prescutum straight; marginal setae of scutellum stand at unequal intervals; third and fourth longitudinal veins divergent near the apex of the wing (Fig. 2H, I) **R. intermedia** Jobling.

Raymondia huberi Frauenfeld, 1855

Strebla huberi Kolenati, 1856.
Strebla africana Kolenati, 1857 (*nec* Walker, 1849).

This species has a very wide distribution in Africa, but outside of this continent it has been found only at Sheikh, Aden. Its distribution extends from Egypt to South Africa, where it has been taken off *Hipposideros caffer* Sundevall, from Zululand; from an undetermined bat at Onderstepoort; and from *Hipposideros caffer* Sundevall and *Cloeotis percivali australis* Roberts, in Transvaal (Bedford, 1932 and 1936). It has been recorded from West Africa, off *H. fuliginosus* Temminck, at Old Calabar, South Nigeria; from undetermined bats, from Abyssinia, Kenya, Belgian Congo and South Rhodesia. Off *H. caffer* Sundevall, from British East Africa; off *Triaenops persicus* Dobson and *Coleura arfa* Peters, from German East Africa; off *Cardioderma cor* Peters, from Solomona Habesch; and *Asellia tridens* Geoffroy, from Egypt. This year it has been recorded by G. H. E. Hopkins, off an undetermined bat collected by him on Mt Elgon, 6300 ft., near Kapretwa, Kenya.

The type specimen was collected by Frauenfeld, in Egypt, and is preserved in the Vienna Museum.

Raymondia setosa Jobling, 1930

Raymondia huberi setosa Jobling, 1930.

This species was described by me as a subspecies of *R. huberi*, but after re-examination of the specimens I have decided to give it a specific name. The form of its thorax and the chaetotaxy of the mesonotum differ distinctly from those of *R. huberi*. The type and the paratype, both males, from an undetermined bat, from Aden, are preserved in the collection of the British Museum (Natural History).

Raymondia planiceps Jobling, 1930

There are four records of this species: off *Cardioderma cor* Peters, from Kenya; from an undetermined bat, from British East Africa; Nairobi; and Krugersdorp District, Transvaal. The holotype female, from British East Africa, is in the collection of the British Museum (Natural History).

Raymondia alulata Speiser, 1908*b*

Raymondia bedfordi Ferris, 1930.
Raymondia quadriceps Jobling, 1930.

The description of Speiser leaves no doubt that the forms described by Ferris and by me are identical with his species. It is the only African species of this genus which has a very distinct alula, and of which the wing venation resembles that of *R. pagodarum* Speiser, as was pointed out by Speiser in his description.

The specimens described by the author of this species were collected by Dr Brauns near Port Elizabeth, Natal, from *Nycteris capensis* Smith; Ferris's specimens were found on *N. damarensis* Peters, from Kaokoveld, South-West Africa; and those described by me were collected in British Somaliland, from an undetermined bat. These last specimens are in the collection of the British Museum (Natural History). They were referred by Kessel (1925) to *Raymondia lobulata* Speiser, which is the Oriental species and does not occur in Africa. Bedford (1936) recorded it from *Petaliadamarensis* Peters (*Petalia* = *Nycteris*), from Gokha Hills, South Africa.

Raymondia waterstoni Jobling, 1931

The type and the two paratypes were taken from *Rhinolophus simulator* Andersen, from Iringa, Uhehe District, German East Africa. These specimens are preserved in the collection of the British Museum (Natural History). The other records are: from an undetermined bat, from Sterkfontein Caves, Transvaal; from *Rhinolophus augur geoffroyi* Anders, from Pretoria; and from *R. empusa* and *Cloeotis percivali australis* Roberts, near Krugersdorp, Transvaal.

Raymondia intermedia Jobling, 1936

The type specimen, female, was collected by Mr A. Loweridge, from *Rhinolophus hildebrandti* Andersen, in Rutschurie, Belgian Congo, and is in the collection of Dr J. Bequaert. Dr F. W. Edwards found the specimens of this species on *R. lobatus* Peters, which he collected during his expedition to Mt Elgon, at 6500 ft., Kenya side. This year Dr G. H. E. Hopkins has collected the specimens of this species from an undetermined bat caught by him on Mt Elgon, near Kapretwa.

ASCODIPTERINAE

General morphology of the genus Ascodipteron

According to Muir (1912) both sexes of this insect, when newly emerged from the puparia, do not differ much from the other Streblidae, and have fully developed wings and legs. When the female finds the host, with its enormously developed proboscis it begins to pierce the skin of the bat and drags itself into the wound, where after losing its legs and wings it becomes encysted. Its abdomen begins to grow round the thorax and the head, so that in a comparatively short time it acquires a flask-shaped form. The growth of the abdomen

probably begins after the first blood-meal, but at what particular moment the female loses its legs and wings has not yet been observed.

According to Muir the male remains an external parasite; its proboscis is very small, resembles that of *Nycteribosca*, and is not adapted for the same purpose as that of the female.

On thirteen bats examined I have found the following infestations: five with one, six with two, one with three, and one with six parasites. They were all embedded near the base of the ears; six parasites were very close together, between the ears. A very careful examination of the bats and the spirit in which they were preserved, revealed not a single male. This seems to indicate that the males have a very short life. The parasite produces a considerable swelling in the skin of the bat, but the only part of it which is visible externally is the posterior, knob-like end of the abdomen (Fig. 4 A, *post.e.*). When the parasite is dissected out of the skin, it looks more like a parasitic worm than a fly. Its flask-shaped body is smooth, with a very distinct constriction between the setose, posterior end of the abdomen and its major, pyriform part which lies inside the skin of the bat. The diameter of this constriction corresponds to that of the circular hole in the skin of the bat, through which the knob-like posterior end of the abdomen protrudes. In the middle of its anterior end (*ant.e.*) there is an opening formed by the folds of the wall of the outgrown abdomen. This leads into the chamber where lie the proboscis, head and the thorax. In all the specimens which were examined, only the most anterior teeth of the proboscis were seen protruding between the folds of the opening.

The knob-like posterior end of the abdomen is rounded and is slightly depressed in the middle (Fig. 4 B). It bears two sclerotized rings in the middle, from which arise many thin setae. Ventrally to these lies the slit-like aperture of a shallow, pocket-shaped chamber, into which open the narrow rectum (*r.*) and the broad vagina (*v.*). The posterior end bears six abdominal spiracles (*ab.s.*), of which four lie dorsally and the other two laterally to the slit-like aperture. Each spiracle is connected with the abdominal trachea by a thin-walled atrium, to which is attached a long, thin, curved rod of the closing apparatus. These rods are shown on the drawing by dotted lines. Externally to these structures the posterior end bears groups of setae, which are attached to very large, projecting thecae. Towards the periphery these setae become more numerous, and are arranged in more or less circular rows.

In order to get a better idea about the characters of the species, I have compared the African specimens with one from China and with another from the Philippines. It has been found that the structure of the posterior end of the abdomen of the Chinese specimen does not differ very much from that of the African one, whereas the Philippine specimen has very weak setae, and owing to the absence of the pocket-shaped chamber, its rectum and vagina open separately.

When the head and the thorax are dissected out of the anterior chamber of the abdomen, they appear as in Fig. 3 A. Of these parts of the body, the most

anterior is an enormously developed theca of the labium (*th.*). In form it is a slightly conical and flattened tube, of which the posterior part is as broad as the head to which it is attached by the membrane. Its anterior part is striated and its surface bears many black, peg-like setae, which are arranged in rows

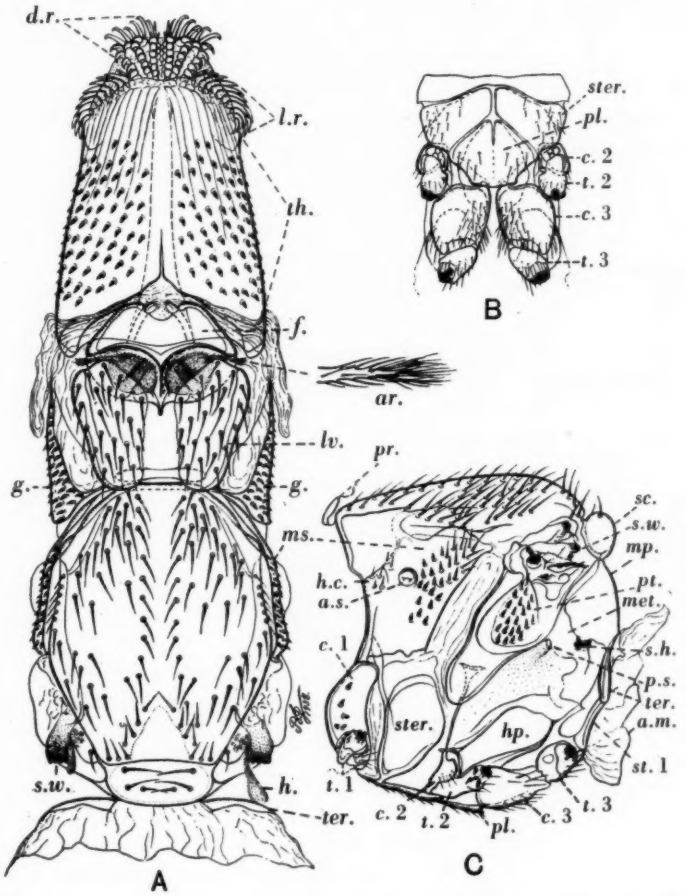

Fig. 3. *Ascodipteron africanum* sp.n. A, proboscis, head and thorax; B, ventral view of thorax; C, lateral view of same. All figures ×85.

and are directed posteriorly. The anterior margin of the theca is connected by the membrane of the greatly reduced labella with that of the labial gutter, which is shown on the drawing by two dotted lines. The labial gutter lies inside the theca, through which it extends to the pharynx. In its lower part is enclosed the hypapharynx, and in its postero-dorsal part lies the short labrum-epipharynx. A more complete description of these parts was given by Muir.

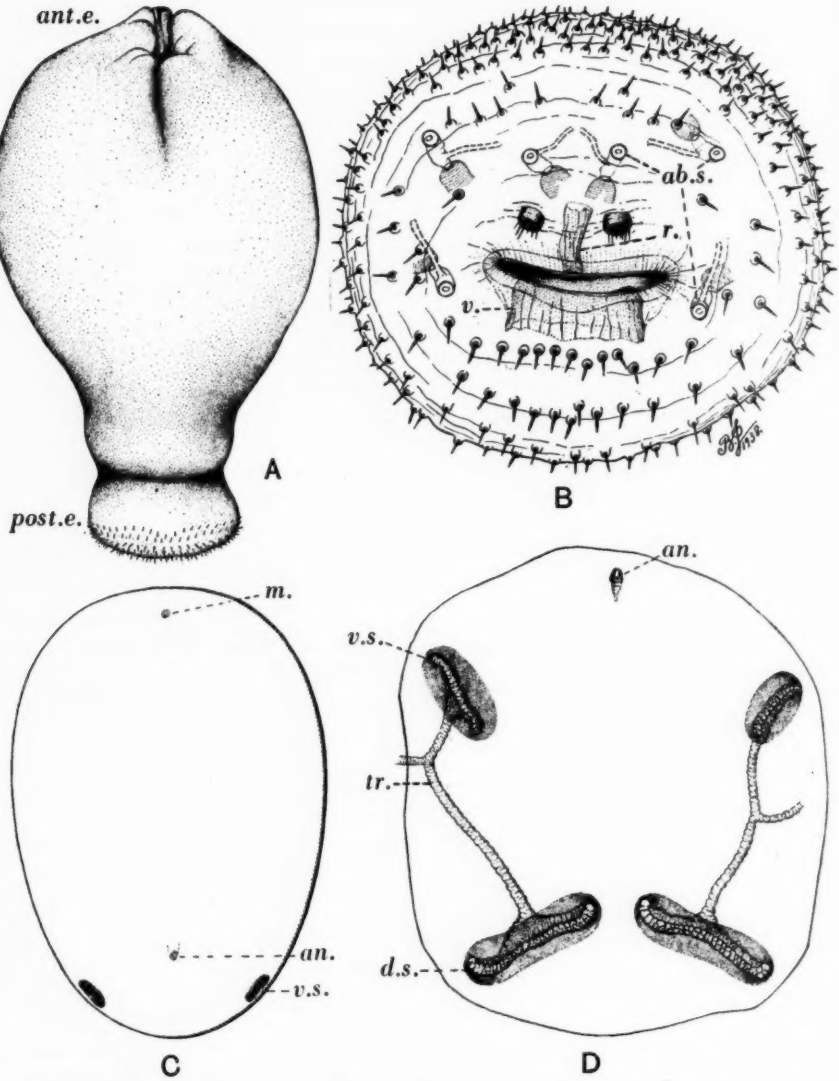

Fig. 4. *Ascodipteron africanum* sp.n. A, female dissected out of skin of bat: *ant.e.* anterior end; *post.e.* posterior end, ×24; B, posterior end of abdomen of female, showing position of six abdominal spiracles (*ab.s.*) and pocket-shaped chamber into which open rectum (*r.*) and vagina (*v.*), ×67; C, ventral view of full-grown larva: *m.* mouth; *an.* anus, ×45; D, posterior end of larva, showing position of ventral (*v.s.*) and dorsal (*d.s.*) spiracles, (*tr.*) tracheae, ×100.

Their structure is the same as that of the other Streblidae which were studied by me (1929).

The membrane of the labella, which connects the anterior margin of the labial gutter with that of the theca, bears fourteen rows of strongly sclerotized teeth: six dorsal (*d.r.*), four lateral (*l.r.*) and four ventral which are not seen on the drawing. Each dorsal row bears long, thin teeth near the labial gutter, and claw-shaped, short ones between these and the dorsal margin of the theca. The teeth of the lateral rows are flat and are more or less falciform, whereas those of the ventral rows resemble the claw-shaped teeth of the dorsal rows. The teeth are protracted and retracted by the movement of the labial gutter, and in the resting position they lie inside the anterior part of the theca.

Owing to the endoparasitic mode of life, the head of *Ascodipteron* has been greatly modified. Its different sclerotized regions have been reduced and separated from each other by the membrane, which makes this region of the body very flexible and facilitates the movement of the proboscis and its protraction from the abdominal chamber.

The most anterior sclerite of the dorsal surface of the head is the frons (*f.*),[1] which lies immediately behind the theca. Structurally it has been very little modified from that of the other Streblidae and resembles the frons of *Nycteribosca*. Its anterior part is strongly sclerotized and is slightly convex; the posterior part forms two deep pits in which lie the antennae. Each antenna is composed of the two visible segments, the surfaces of which bear numerous scales. The cup-shaped first segment, or pedicle, bears one dorsal setae and partly conceals the second segment, or flagellum. The latter segment is ovoid. It has many sensory pits and bears the branching arista (*ar.*), which projects laterally between the posterior margin of the anterior part of the frons and the anterior margin of the laterovertex. The true first segment of the antennae, or scape, is fused with the posterior part of the antennal pit and is invisible on the total preparation of the head. Behind the frons and overlapping the antennae lie two lateroverticles (*lv.*). Each of them is a thin plate which bears many moderately long setae. Laterally to the lateroverticles and separated from them by a very broad membrane lie the genae (*g.*). They are very strongly sclerotized and bear many black setae similar to those arising from the theca. On the ventral surface of the head the genae merge into the narrow post-genae which are devoid of setae. The structure of the post-genae varies in the different species. In some they are fused together, but in others they are separated from each other by a very narrow membrane. Owing to the great reduction of the occipital region, the posterior foramen of the head is as broad as the anterior foramen of the thorax, with which it is closely connected by a very short membranous joint.

The thorax shows considerable variation in its structure in the different

[1] In my previous papers, where I used the terminology of Peterson (1916), this region of the head was termed the fronto-clypeus, but in fact, in all the Schizophora, it represents only the frons. Their clypeus is greatly reduced and forms the anterior part of the fulcrum.

species. In the African species it is higher than long, strongly compressed laterally, and its prothorax (*pr.*) is separated from the prescutum; whereas in the Philippine specimen it is slightly longer than high, with the prothorax fused with the anterior margin of the prescutum. The prescutum and the scutum form a slightly convex, octagonal plate which bears moderately strong setae. The arrangement of these setae varies in the different species of this genus. The scutellum (*sc.*) is oval when examined from above, with a few setae.

The pleural part of the thorax is composed of the same sclerites as that of the other Diptera Cyclarrhapha, but their position and the structure are very much altered owing to the modification of the form of the thorax. The most anterior sclerite of this part of the thorax is the irregularly shaped mesopleuron (*ms.*), which extends from the lateral margin of the mesonotum to the front coxa (*c.*1). Its anterior part is fused with the humeral callus (*h.c.*) which bears a few very small setae. Behind the humeral callus lies the anterior spiracle (*a.s.*), and behind this the mesopleuron bears a group of black, peg-like setae. The modification of these setae from the long ones can be easily followed in the dorso-posterior part of this sclerite, which lies anteriorly to the base of the wing stumps. The posterior margin of the mesopleuron is separated from the pteropleuron (*pt.*) by a broad membrane, which corresponds to the mesopleural suture of the other Diptera. The pteropleuron is a long sclerite extending from the base of the wing to the dorso-posterior part of the sternopleuron (*ster.*). In its dorsal part it has a patch of setae similar to those arising from the mesopleuron. Behind the pteropleuron there lies the irregularly shaped metapleuron (*met.*), which carries the stump of the halter (*s.h.*). In the corner formed by this sclerite and the posterior margin of the pteropleuron there is present the posterior thoracic spiracle (*p.s.*). This is much smaller and not so distinct as the anterior spiracle. Below the scutellum and the base of the abdomen lies the mesopostnotum (*mp.*). It is strongly convex and has the same structure as that of the other Streblidae. The hypopleuron (*hp.*) lies above the coxa of the hind leg, being separated from the other sclerites by the partly sclerotized membrane.

The ventral wall of the thorax (Fig. 3B) is formed by the sternopleura (*ster.*) and the pleurotrochantines (*pl.*). The last-named sclerites are fused together. They are separated from the sternopleura by a Λ-shaped suture, of which the lateral branches extend to the base of the coxae of the middle legs (1, 2).

The loss of the legs in the females of *Ascodipteron* occurs, as a rule, at the junction of the trochanter with the femur. The resulting wound is healed by the formation of a very darkly pigmented cuticular plug, which neither dissolves nor discolorizes during the prolonged boiling of the specimen in caustic potash. The remaining joints of the legs have the same structure as those of the other Streblidae, with the exception of the coxae of the forelegs, which have become flattened and displaced laterally.

The membranous abdomen, which has already been described, is attached to the posterior foramen of the thorax, which is formed by the reduced first and second tergites (*ter.*) and the first sternite (*st.* 1).

THE LIFE HISTORY AND THE STRUCTURE OF THE LARVA OF *ASCODIPTERON AFRICANUM* sp.n.

(Fig. 4C, D)

The life history of *Ascodipteron* was described for the first time by Muir. According to him the female produces fully grown last stage larvae, one at a time, as do the other pupiparous flies. The larva is 1·5 mm. long and 1 mm. across the largest diameter. When it is ejected from the uterus and drops to the ground, it immediately forms the puparium. The adult fly emerges in from 30 to 33 days after the ejection of the larva.

During the present study two fully grown larvae were dissected from the uterus. They were both about the same size and form as those which were obtained by Muir. Their length was 1·5 mm. and the width 1·1 mm. across the largest diameter. The ventral surface of the larva is almost flat, whereas the dorsal one is strongly convex in its anterior half. The cuticle of the larva is very smooth and is without any traces of segmentation, or grooves. The mouth of the larva (*m.*) is a very tiny orifice surrounded by slightly thickened and very lightly pigmented cuticle. It lies in close contact with the duct of the accessory glands, from which the larva receives its nourishment. The anus (*an.*) is a sclerotized ring to which is attached the thin-walled rectum. On the preparation it is more distinct than the mouth, and lies in the posterior part of the ventral surface of the larva.

The most conspicuous organs of the posterior part of the larva are the four spiracles. They may be conveniently divided, according to their position, into two pairs: the dorsal, and the ventral. The dorsal spiracles (*d.s.*) lie close together in the postero-dorsal part of the larva, not far from the posterior end. They are larger than the ventral ones, and their peritreme is slightly narrower in the middle part than near their rounded ends. The ventral spiracles (*v.s.*) have an oval peritreme and they lie on the ventral surface, posteriorly to the anus. The peritreme of the spiracles is a thin, darkly pigmented plate, in the middle of which lies the curved spiracular slit. The spiracle is guarded against the entrance of foreign particles by a network of sclerotized processes, which arise from the inner wall of the slit. From the internal margin of the slit arises a thin-walled atrium. The atrium of each dorsal spiracle is connected by a thick trachea with that of the ventral spiracle which lies on the same side of the larva. From the middle of each of these tracheae arises the longitudinal branch, which passes through the body of the larva anteriorly, giving off lateral branches.

The systematic position and the definition of the genus *Ascodipteron*

The genotype of this insect, the endoparasitic female, from Java, was described by Adensamer (1896), under the name of *A. phyllorinae*. The author indicated its relation to the Pupipara, and believed that its male must be free-living on bats.

In 1898, Monticelli described another species, *A. lophotes*, from Eritrea, and created for this genus a new family Ascodipteridae.

Muir (1912) referred the genus to the Streblidae. He pointed out in his paper that the male of *Ascodipteron* is so similar in its structure, habit and development to the Streblidae that it is difficult to separate it from that family.

By Falcoz (1926) these insects were referred to Ascodipterinae, to which subfamily they were also referred by me (1936).

The following description of the genus is based upon the examination of the structure of the females of the three different species. The characters of the male are not included in this description, because it has not been studied completely.

Genus **Ascodipteron** Adensamer, 1896

Body flask-shaped, its posterior, knob-like end setose, with six abdominal spiracles and two setose plates, or cylindrical sclerites in the middle; rectum and vagina separate, or end inside a pocket-shaped chamber which opens by a transverse slit-like aperture. Proboscis, head and thorax concealed in the chamber formed by the abdominal wall. Theca of labium longer than the head and as broad basally as the head, with fourteen rows of strong teeth arising from the membrane of its distal end; maxillary palps absent. Sclerotized regions of head separated by membrane; eyes absent; antennae two-segmented, with dorsal branching arista. Thorax as long as or shorter than high, compressed; mesonotal suture absent; scutellum small, oval, or more or less crescent-shaped. Wings, halteres and legs cast off, leaving stumps of the first two and coxae and trochantines of the last named. Endoparasites of skin of bats.

Ascodipteron africanum sp.n.

(Figs. 3, 4)

Body of the endoparasitic female 3·5 mm. long and 2·7 mm. across the largest diameter. Posterior end of abdomen bearing many darkly pigmented setae arising from elevated thecae; rectum and vagina opening into a pocket-shaped chamber, which has a transverse, slit-like aperture; dorsally to this aperture there are present two small, cylindrical sclerites, the external ends of which bear fine setae; abdominal spiracles presenting no distinctive characters.

Theca of labium slightly conical, 0·5 mm. long and 0·4 mm. wide in its basal part (Fig. 3A, *th.*), its postero-dorsal margin broadly emarginated and enclosing the anterior part of frons; it bears many black, peg-like setae arranged in rows. Teeth of the labellar membrane presenting no distinctive characters. Frons as figured (*f.*), without setae; distal end of antennal arista with many branches (*ar.*); lateroverticcs (*lv.*) irregularly shaped plates, with very thick outer margin; they bear many lightly pigmented moderately long setae, and their anterior parts overlap the antennae; genae (*g.*) more or less triangular, with many black, peg-like setae; post-genae without setae, separated from each other by narrow membrane.

Thorax higher than long, strongly compressed; mesonotum convex, as long as the theca of the labium; prescutum and scutum fused together, forming an octagonal plate, which bears many setae similar to those arising from the lateroverticcs in its antero-lateral and postero-lateral parts, and two rows of the same setae along the middle; scutellum oval, with four long and two shorter setae; humeral calluses (*h.c.*) with four small setae; mesopleuron (*ms.*) with a patch of black, peg-like setae behind the rounded anterior spiracle and lightly pigmented, long setae in the dorso-posterior part; pteropleuron (*pt.*) as figured, with a patch of peg-like setae below the wing stumps; hypopleura (*hp.*) and the lateral parts of sternopleura (*ster.*) without setae.

Ventral surface of thorax flat, with a few very thin setae in its lateral and postero-lateral parts (Fig. 3B); the ventral parts of sternopleura separated from each other and from the pleurotrochantines (*pl.*) by Λ-shaped suture. Coxa of forelegs a flat curved plate, which bears a few small, peg-like setae; coxa of middle legs ring-shaped, with a few very small, thin setae; coxa of hindlegs enlarged, longer than broad, with a patch of thin setae on the inner half and with a few setae along the postero-lateral margin; trochantines of the fore and middle legs with some small, peg-like, and a few very thin, short setae; those of the hindlegs with thin setae only.

Type and many paratypes, all females, are preserved in the collection of the British Museum (Natural History). They were found on bats, *Miniopterus natalensis arenarius* Heller, which were collected by Dr F. W. Edwards, in 1935, and by Dr G. H. E. Hopkins, this year, near Kapretwa, Kitale, Kenya.

Ascodipteron lophotes Monticelli, 1898

Two specimens, females, were found on *Rhinolophus clivosus* Cretzschmar, from Assab, Eritrea, East Africa. In his paper the author describes the anatomy and the morphology of this form, but does not give a separate diagnosis of this species.

Ascodipteron tabulatum Speiser, 1908*a*

One male specimen, from the skin of an undetermined species of bat of the genus *Miniopterus*, from Sakana, East Madagascar. The characters employed by the author of this species are mostly generic.

Appendix. Amendment of name

In my paper published in *Parasitology*, 1933, **30**, 381, I placed the name *Trichobius kesseli* Guimaraes (1938) in the synonymy of *T. parasiticus* Gervais (1844); but on p. 377 of the same paper I have used the feminine form of the same name, *T. kesselae* for a new species. As according to the *Rules of Zoological Nomenclature*, art. 36, it is not permissible to employ a name once rejected, I take this opportunity to amend the name of *T. kesselae* to *T. pseudotruncatus* sp.n.

REFERENCES

Adensamer, T. (1896). Über *Ascodipteron phyllorinae* (n.gen., n.sp.) eine eigenthümliche Pupiparenform. *S.B. Akad. Wiss. Wien*, Abt. 1, **10**, 400.

Bedford, G. A. H. (1932). A synoptic check-list and host-list of the endoparasites found on South African Mammalia, Aves and Reptilia. *18th Report of the Director of Veterinary Services and Animal Industry*, Union of South Africa.

—— (1936). Supplement No. 1. *Onderstepoort Journal of Veterinary Science and Animal Industry*, **7**, 69.

Falcoz, L. (1923). Biospheologica. No. XLIX. Pupipara (Diptères). *Arch. Zool. Exp. gén.* **61**, 521.

—— (1924). *Diptères Pupipares du Muséum National d'Histoire Naturelle de Paris*, **30**, 1.

—— (1926). *Diptères Pupipares. Faune de France*, p. 14. Paris.

Ferris, G. F. (1930). Some African Diptera Pupipara. *Parasitology*, **22**, 275.

Frauenfeld, J. (1855). Über eine neue Fliegengattung *Raymondia* aus der Familie der Coriaceen, nebst Beschreibung zweier Arten derselben. *S.B. Akad. Wiss. Wien*, **18**, 320.

Jobling, B. (1929). A comparative study of the structure of the head and mouth-parts in the Streblidae (Diptera Pupipara). *Parasitology*, **21**, 417.

—— (1930). A revision of the genus *Raymondia* Frauenfeld (Diptera Pupipara). *Parasitology*, **22**, 283.

—— (1931). A new species of the genus *Raymondia* Frauenfeld (Diptera Pupipara, Streblidae) with a note on *Raymondia quadriceps* Jobling and *R. bedfordi* Ferris. *Parasitology*, **23**, 79.

—— (1934). A revision of the genus *Nycteribosca* Speiser (Diptera Pupipara, Streblidae). *Parasitology*, **26**, 64.

—— (1936). A revision of the subfamilies of the Streblidae and the genera of the subfamily Streblinae (Diptera Acalypterae) including a redescription of *Metelasmus pseudopterus* Coquillett and a description of two new species from Africa. *Parasitology*, **28**, 355.

Kessel, Q. C. (1925). A synopsis of the Streblidae of the world. *New York ent. Soc.* **33**, 11.

Kolenati, F. A. (1856). *Die Parasiten der Chiropteren*. Brünn.

—— (1857). *Die Parasiten der Chiropteren*. Dresden.

—— (1863). Beiträge zur Kenntniss der Phthiyrio-myiarien. *Horae Soc. Ent. Rossicae*, **2**, 11.

Monticelli, F. S. (1898). Un altro specie del genere *Ascodipteron*, parassita del *Rhinolophus clivosus* Ropp. *Ric. Lob. Ant. norm. Univ. Roma*, **6**, 4, 201.

Muir, F. (1912). Two new species of Ascodipteron. *Bull. Mus. comp. Zool. Harv.* **54**, 351.

Peterson, A. (1916). The head-capsule and mouth-parts of Diptera. *Illinois Biol. Monogr.*, iii, No. 2.

SCHINER, J. R. (1868). Diptera. *Reise der Novora*, Zool. Theil. Abt. B, p. 375.

SPEISER, P. (1900). Über die Strebliden, Fledermausparasiten aus der Gruppe der Pupiparen Dipteren. *Arch. Naturgesch.* **66**, 31.

SPEISER, P. (1908*a*). Die Diptera pupipara der madagassisch-maskarenischen Region. (In *Alfred Voeltzkow. Reise in Ostafrika in den Jahren* 1903–5, Publ. 1906–10, p. 202.)

—— (1908*b*). Diptera pupipara (Hippoboscidae). *Denkschr. med.-naturw. Ges. Jena*, **13**, 175.

WALKER, F. (1849). *List of the Species of Diptera Insects in the Collection of the British Museum*, part N, p. 1146.

Explanation of reference lettering

ab.s. abdominal spiracles; *a.m.* abdominal membrane; *an.* anus; *ant.e.* anterior end of abdomen; *ar.* arista; *a.s.* anterior thoracic spiracle; *c.* 1, coxa of forelegs; *c.* 2, coxa of middle legs; *c.* 3, coxa of hindlegs; *d.r.* dorsal rows of teeth; *d.s.* dorsal spiracles of larva; *f.* frons; *g.* gena; *h.* halter; *h.c.* humeral callus; *hp.* hypopleuron; *l.r.* lateral rows of teeth; *lv.* laterovertices; *met.* metapleuron; *mp.* mesopostnotum; *ms.* mesopleuron; *pl.* pleurotrochantines; *post.e.* posterior end of abdomen; *pr.* prothorax; *p.s.* posterior thoracic spiracle; *pt.* pteropleuron; *r.* rectum; *sc.* scutellum; *s.h.* stump of halteres; *st.* 1, first sternite; *st.* 2, second sternite; *ster.* sternopleuron; *s.w.* stumps of wing; *th.* theca of labium; *ter.* tergite; *tr.* trachea; *t.* 1, trochanter of foreleg; *t.* 2, trochanter of middle leg; *t.* 3, trochanter of hindleg; *v.* vagina; *v.s.* ventral spiracles of larva.

(*MS. received for publication* 9. XII. 1938.—Ed.)

ANCHISTROTOS ZEUGOPTERI (T. SCOTT)

A PARASITIC COPEPOD OF *ZEUGOPTERUS PUNCTATUS*

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.)

(With 3 Figures in the Text)

Habitat and material. About a hundred specimens including both sexes were taken on the upper surface, especially the dorsal and ventral fins, of two *Zeugopterus punctatus* (Bloch), 13 and 21 cm. respectively in length, by Miss Nora G. Sproston at Roscoff in September 1938.

Record. The genus *Anchistrotos* was erected by Brian 1906 (*A. gobii* on *Gobius capito*) for an ergasilid copepod resembling *Bomolochus*, but which, in the female, presented two striking differences from the latter genus:

(1) The *maxillipedes*, instead of being placed laterally to the maxillae as in the females of *Bomolochus*, are located posteriorly to them as in males (Fig. 1).

(2) A *maxillary hook* is present (Figs. 1 and 2, H).

On examination of a new species, *A. laqueus* Leigh-Sharpe, 1935, from Plymouth, were added:

(3) *Cephalothorax* bluntly rounded anteriorly with a rounded median projection.

(4) *Abdomen* of four segments (Fig. 1) (three in the male).

To these may be added, on consideration of this species, as common to all three:

(5) *Antennules* six-articled.

(6) Eggs few and large, especially in *A. laqueus*.

Body (Fig. 1). *Cephalothorax* orbicular, rounded in front, the anterior median projection not very pronounced. *Third thoracic* segment only half as wide as the greatest width of the cephalothorax; the others diminishing regularly in size. *Genital segment* much longer than wide. *Abdomen* of four segments in the female, but three in the male, decreasing in size posteriorly; abdominal appendages elongated, each bearing terminally two very long and one outer shorter setae, and some small lateral ones. There is a well-marked dorsal pair of pink eyes.

Appendages (Figs. 1, 2). *Antennules* six-articled, the three proximal articles densely setose, widened and flattened; the three distal articles small with a few setae, terminal article with three large and one small setae. *Antennae* four-articled; the fourth or proximal article having been overlooked in the previous species since the others were folded over it, the next article as long as the other two together which are flexed upon it, antipenultimate article with one long seta, distal article terminating in three claws.

The *maxillary hook* is smooth, elongated and curved, as in *Caligus*, and not stout and abruptly hooked as in *A. laqueus*; its point of attachment is not anterior

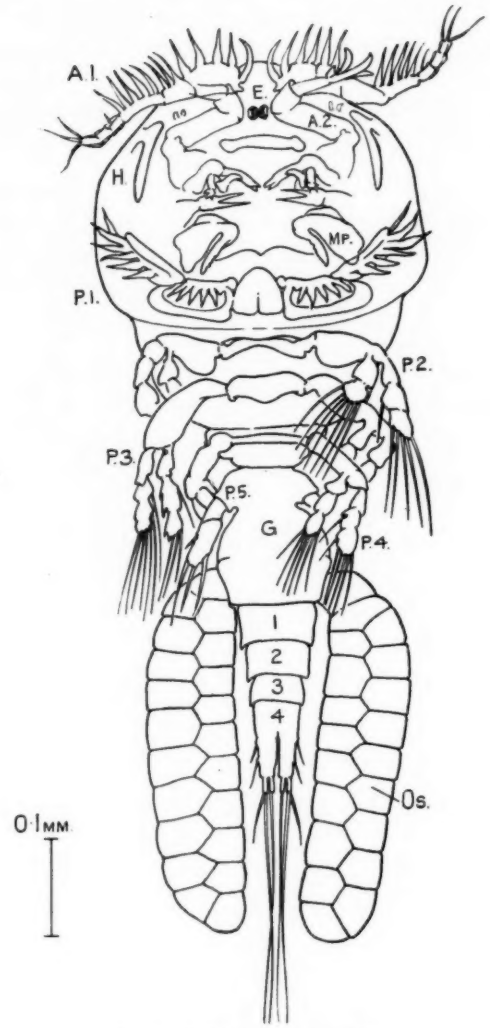

Fig. 1. *Anchistrotos zeugopteri* ♀.

and basal, but lateral. Anterior to it are two minute refringent projections, irregularly-shaped and varying in different specimens or even in the two pairs of the same specimen. In the female the hook is a little more than three times as long as in the male.

Mandibles with a large base, undivided distally and ending in two subequal claws.

First maxillae three-articled; the distal article is small and bears three short setae; the basal article has the usual curved hook.

Second maxillae two-articled, ending in two long spines, the posterior of which is toothed.

Maxillipedes immediately posterior to the maxillae, instead of being lateral to them, in both sexes; no distinct articulations, the faint indications

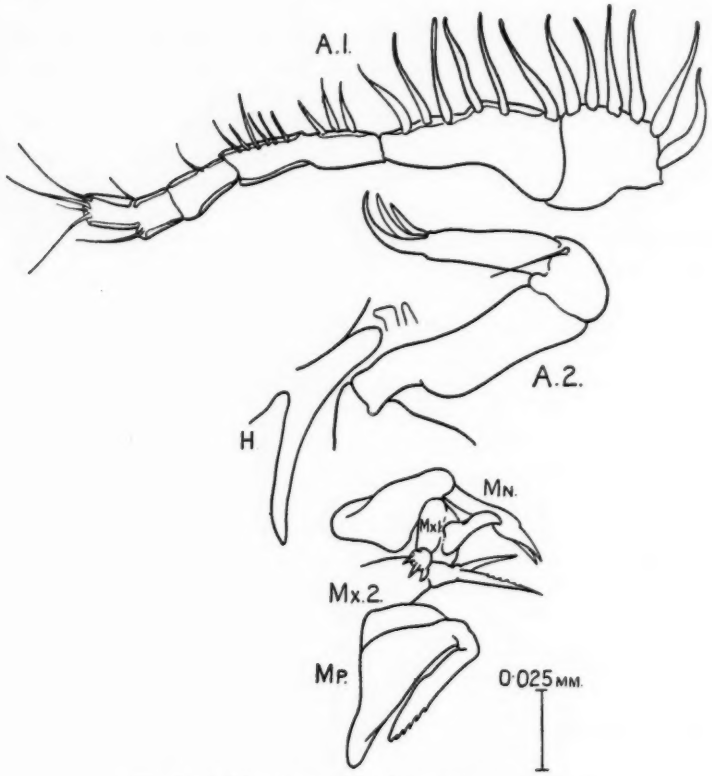

Fig. 2. *Anchistrotos zeugopteri* ♀, anterior appendages.

of four seen in *A. laqueus* not visible here. From the enlarged base arises an abruptly bent stout spine serrated along one edge. In the male the maxillipedes are very large and cover the buccal appendages, and the stout spine is replaced by a long curved claw serrated along one edge (Fig. 3). The maxillipedes in the males that I have examined do not appear to be symmetrical, one being larger and having a differently-curved claw. The usual two accessory setae are not present in either sex.

Four pairs of *Pereiopods* biramous, each ramus three-articled, except in the first pair which is flattened, bears broad setae, and has each ramus two-articled.

Fifth pair of *Pereiopods* uniramous, two-articled, the long distal article flattened and bearing three apical and one lateral setae.

Ovisacs twice as long as abdomen without its caudal setae: eggs large, about eleven in a row.

Remarks. The male has not been previously described.

I have examined eight, about half, of T. Scott's type specimens of *Bomolochus zeugopteri* 1902, from the Clyde estuary, in the British Museum (Natural History) and find that they agree with the foregoing account rather than with his own description. He has miscounted the number of segments in the abdomen, and

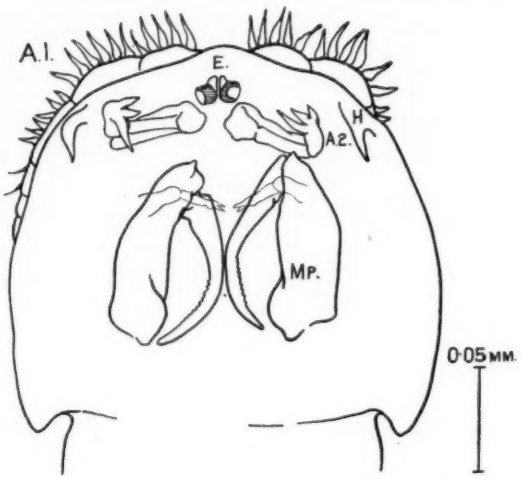

Fig. 3. *Anchistrotos zeugopteri* ♂, cephalothorax.

articles in the antennule, and overlooked the maxillary hook. They must be placed in the genus *Anchistrotos* since they show four segments in the abdomen, the presence of a maxillary hook, and the maxillipedes are placed posteriorly to the buccal appendages instead of laterally. The maxillipedes are as I have figured them (Fig. 2), and in a cleared specimen I failed to discern the two setae on the serrated spine, a situation from which they would easily be detached during the functioning of the appendage.

Scott's specimens differ from mine in being larger; the antennules and pereiopods are more profusely setose; the abdominal appendages each bear four setae; the eggs are large, about twelve in a row, which is almost as he figures them; the first pereiopods stand out at right angles well beyond the cephalothorax so that they are more easily seen; the maxillary hook is smaller and more curved, as in males (Fig. 3). These small differences, except the last, can be explained by the suggestion that Scott's specimens are older. While a

divergence of locality may also account for some of these differences, I cannot consider them sufficiently important to warrant the erection of a new species.

Numerous examples of *A. laqueus* were taken on *Serranus cabrilla* by Dr C. L. Oakley at Plymouth in July 1937, agreeing in every particular with my previous description. Wilson (1932, p. 385) is mistaken in giving as a generic character for *Anchistrotos* that the ramus of the fifth pereiopod has but one article, as it has two in the species I have described whatever may be the case in *A. occidentalis* Wilson.

REFERENCES

Brian (1906). *Copepoda parassiti dei Pesci d'Italia.* Genova.

Leigh-Sharpe (1935). *Anchistrotos laqueus* n.sp., a parasite copepod of *Serranus cabrilla*. *Parasitology*, **27**, 270.

Scott (1902). Notes on some parasites of fishes. *20th Ann. Rep. Fishery Board for Scotland*, pp. 288–99, pls. XII, XIII.

Scott & Scott (1913–14). The British parasitic copepoda. *The Ray Soc.* London. (A copy of the above.)

Wilson (1932). The copepods of the Wood's Hole Region, Mass. *Bull. U.S. Nat. Mus. Washington*, No. 158.

EXPLANATION OF THE LETTERING OF FIGURES 1–3

1–4 (Fig. 1) = abdomen; *A.* 1 = antennules; *A.* 2 = antennae; *E* = eyes; *G* = genital segment; *H* = maxillary hook; *Mn.* = mandible; *Mp.* = maxillipede; *Mx.* 1 = first maxilla; *Mx.* 2 = second maxilla; *Os.* = ovisacs; *P.* 1–5 = pereiopods.

All drawn with the camera lucida.

(*MS. received for publication* 5. xii. 1938.—Ed.)

NOTE ON THE PIGMENTATION OF *LERNAEOPODA* (COPEPODA)

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.)

(With 1 Figure in the Text)

I am greatly indebted to Prof. O. Nybelin of Göteborg for the loan of two specimens of female *Lernaeopoda scyllicola* Leigh-Sharpe, 1915 taken by him from the extra-cloacal region of two *Scylliorhinus canicula* (L.) during the first fortnight of September 1938 at Roscoff which show peculiar cases of pigmentation (Fig. 1).

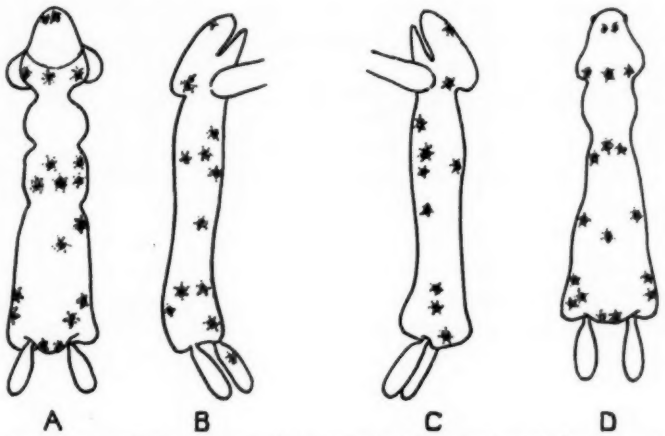

Fig. 1 *Lernaeopoda scyllicola* ♀. A, one of the two pigmented specimens in dorsal aspect; B, the same in right lateral aspect; C, the same in left lateral aspect; D, the other specimen.

Three other specimens taken from the same region of the like host during the same period at the same locality showed no traces of pigmentation, but were normal.

The pigmentation takes the form of a number of large bright brownish red spots, which, under magnification, appear stellate, almost, but not completely, symmetrically arranged. A common feature seems to be three spots at the posterior end of the cephalothorax, in one case there are two additional spots in front, in the other five very small ones.

There are certain areas on the trunk, towards the hinder end of which only is a slight asymmetry noticeable, and in one case on an abdominal appendage. Each female is accompanied by a male which is not pigmented.

(*MS. received for publication* 5. xii. 1938. Ed.)

LIFE CYCLE OF *COITOCAECUM ANASPIDIS* HICKMAN, A NEW ZEALAND DIGENETIC TREMATODE

By W. V. MACFARLANE

Department of Agriculture, Wallaceville

(With 13 Figures in the Text)

Introduction

While in Europe and America knowledge of trematodes increased towards the end of the nineteenth century and during the twentieth century, practically no work was carried out on those animals in New Zealand. Thomas, who had investigated the life cycle of the liver fluke in England, accepted a chair in Auckland, New Zealand in 1883 (about the same time that the fluke appeared in this country), but he did not further his study of *Fasciola hepatica*.

There is very little recorded knowledge of trematodes. Hutton (1904), however, gives a list of thirty digenea in his Index. The explanation of this record was kindly provided by Dr W. B. Benham: "Rudolphi and others described the flukes from hosts which occur in European waters; but these hosts also occur in and around New Zealand. It is therefore likely that they will harbour the same species of parasites, hence Hutton included them in his Index—perhaps prematurely." The results of this *a priori* zoology have not been examined.

Monticelli (1899) made observations on specimens of *Gyrocotyle rugosa* Diesing and *Distomum veliporum* Creplin which had been sent to the British Museum from Dunedin. In the reports of the Livestock Division of the Department of Agriculture from 1897 onwards there are records of the occurrence and spread of *Paramphistomum cervi* and *Fasciola hepatica* in the country, but nothing was discovered concerning their intermediate hosts.

In 1904 Chilton noted the occurrence of a pectinate cercaria in the gills of *Chione stutchburyi* found at Portobello. Twenty-three years later, Hopkirk (1927) reported that *Potamopyrgus antipodum* and *P. corolla* carried four cercaria in the North Island. These are now recognized to be the parthenita of *Coitocaecum anaspidis*, *Telogaster opisthorchis*, *Opechona stegadexamenes* and cercaria IV of the present series of publications. An account of the two local hosts of *Fasciola hepatica* was published in 1937 by Macfarlane.

There has been, accordingly, an almost untouched portion of the freshwater fauna awaiting examination. In this work there is presented an account of the formal anatomy and development of the trematodes discovered, together

with indications of the functions of the parts. This serves as a prelude to a study of the community relations of the hosts and their parasites—their habitats, food and behaviour.

METHODS

Examination of living trematodes in 0·8 or 0·4 % Ringer was found to be the simplest and best technique. And fully grown metacercariae removed from their cysts with fine needles, were at the most satisfactory stage for the elucidation of genital and excretory details. Reproductive adults were either filled with eggs or with follicles which obscured the organization. Good fixation for sectioning was obtained with osmium tetroxide 0·1 % solution. Bouin and acetic-mercuric chloride satisfactory. Formaldehyde 40 % added to Ringer solution containing swimming cercariae is excellent as a fixative for those animals. Ehrlich's, Delafield's and iron alum haematoxylin were used as basic stains; light green, fuchsin, eosin, nile blue, safranin and orange G as acid cytoplasmic stains. Iron haematoxylin, light green and eosin yield the best preparations.

Intra-vitam staining of all developmental stages and the adult gave admirable results. Methylene blue, neutral red, Janus green and methyl green were employed. In general, methylene blue is more useful than methyl green as a basic stain; and neutral red is invaluable as an index of metabolism.

SYSTEMATIC POSITION AND OCCURRENCE OF *COITOCAECUM ANASPIDIS*

Family: Allocreadiidae Stossich, 1904.
Subfamily: Coitocaecinae Poche, 1925 (Coitocaecidae Ozaki, 1929).
Coitocaecum Nicoll, 1915.
C. anaspidis Hickman, 1934.

Nicoll established the genus in 1915 for *Coitocaecum gymnophallum*. Ozaki added five species and made a new family for them in 1929; four years after Poche established a subfamily for Nicoll's species. Since 1929 Yamaguti (1934) has described a new species, *C. glandulosum*; and Pigulewsky (1931) and Hickman (1934) both made further additions to the genus.

Hickman described his species from progenetic specimens taken from *Anaspides tasmaniae* Thomson. He did not know the adult, nor any of the larval stages other than the miracidium.

In the provinces of Canterbury and Wellington *Coitocaecum anaspidis* is found in the intestine of *Gobiomorphus gobioides*, young *Salmo fario*, *Galaxias brevipennis*, *Galaxias attenuatus* and of eels under 30 cm. in length. Rarely it is found in older eels. Its larval stages occur in *Potamopyrgus* species and in the amphipod *Paracalliope fluviatilis* Thomson.

Structure of different stages of *C. anaspidis*

Adult. The description of the mature metacercaria given by Hickman is adequate; but for completeness it will be repeated briefly, with the addition of an account of the excretory system and of variations occurring in the New Zealand specimens.

The dimensional range of the adult is indicated in Table I. There are no spines in the cuticle nor are there any cuticular glands (Fig. 1). The acetabulum is large and powerful, its centre being a little less than half-way from the anterior end. The oral sucker has half the diameter of the acetabulum. It opens on to a short prepharynx, which is followed by pharynx and oesophagus. The caeca meet ventrally to the excretory vesicle at the posterior end to form a

Table I. *Dimensions of* Coitocaecum anaspidis

	Range of size		Hickman's	Average of 6	
	mm.	mm.	mm.	mm.	Proportion
Length	1·48	0·95	2·40	1·22	100
Breadth	0·47	0·28	0·75	0·41	34
Oral sucker	0·13	0·10	0·16	0·12	10
Acetabulum	0·28	0·18	0·26 ×0·37	0·22	18
Acetabulum from anterior end	0·82	0·38	—	0·62	50
Prepharynx	0·012	0·02	—	0·019	1·6
Pharynx	0·07	0·04	0·08	0·06	5·0
Oesophagus	0·11	0·05	0·15	0·09	8·0
Anterior testis	0·13 ×0·25	0·12 ×0·12	—	0·15 ×0·20	—
Posterior testis	0·22 ×0·26	0·12 ×0·15	0·15 ×0·20	0·13 ×0·21	—
Ovary	0·11 ×0·17	0·046 ×0·06	0·09 ×0·09	0·09 ×0·13	—
Egg	0·051 ×0·087	0·042 ×0·054	0·049 ×0·080	0·049 ×0·077	—

Average excludes Hickman's figures which are taken from the large progenetic individuals and are thus greater than those of a normal adult parasite.

The average lengths or diameters of the organs are expressed in the last column proportionally to the body length.

complete loop. The testes show considerable variation which is correlated neither with host nor with locality (Fig. 4). Commonly they are rectangular and strictly tandem, occupying most of the hind body in the intercaecal region. There is, however, a variant in which the testes are conical and oblique. Between these extremes there are specimens showing rounded rectangular tandem and spherical semi-tandem testes. The vasa deferentia meet immediately before they enter the vesicula seminalis, which opens in the triangle between the oesophagus, the left caecum and the body wall (when the ventral surface is downward). There is a single wall to the vesicle which tapers to a ductus ejaculatorius, and ends in a cirrus which is capable of eversion to 0·32 mm. (Fig. 3). A short cirrus sac containing prostate glands encloses the cirrus and the ductus ejaculatorius, but it does not continue down to enclose the vesicula seminalis.

The ovary lies morphologically to the right of the middle line, that is, on the opposite side to the genital pore. The oviduct leaves the ovary terminally

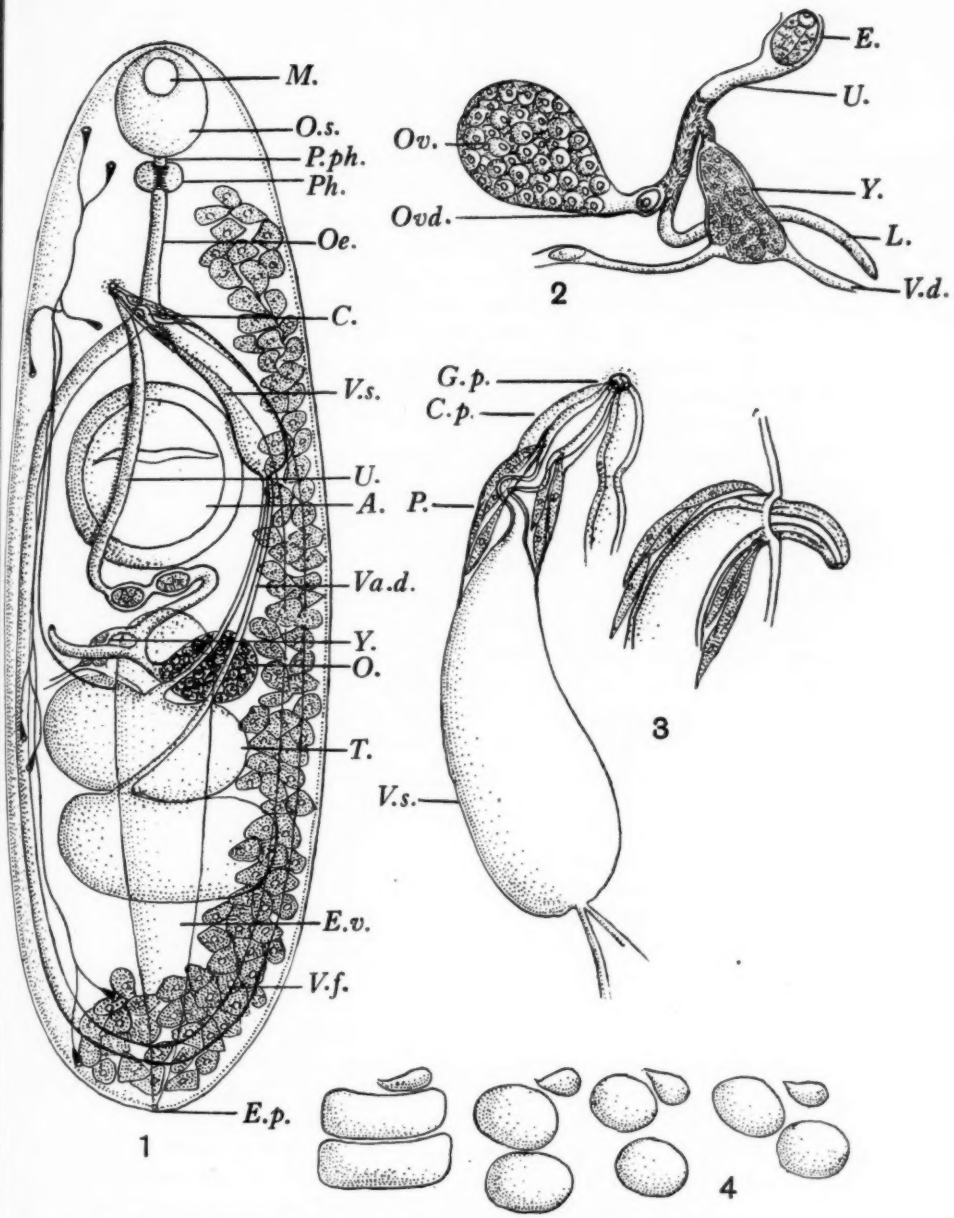

Fig. 1. Adult *Coitocaecum anaspidis*, dorsa surface upwards. Size 1·3 × 0·40 mm.
Fig. 2. Ovary and allied organs from the ventral side.
Fig. 3. Vesicula seminalis, cirrus and metraterm. The same organs shown everted.
Fig. 4. Variations in the shape and proportions of the gonads of the adult.

and shortly joins the Laurer canal, which opens on the dorsal surface. Cilia beat along the uterus and oviduct towards the Laurer canal (Fig. 2). Where the yolk reserve enters the uterus there is a circlet of shell glands. The vitellaria, made up of groups of follicles 0·03 mm. long, extend from the level of the genital pore dorsally along the caeca to meet posteriorly. A duct from each side enters the yolk reserve dorsally. The uterus is short, containing never more than ten eggs. It ends in a metraterm which does not join with the cirrus—the two open side by side in the genital pouch.

The club-shaped excretory vesicle is dorsal to the gonads (Fig. 1). It sometimes reaches beyond the acetabulum, but usually it ends immediately behind that organ. From each side of the vesicle, one-fifth of its length from its anterior end, arise the main collecting tubes. The tubes pass towards the caecum, then turn forward to the level of the genital pore, where they branch into the anterior and posterior ducts. The finer tubules are not well defined, but it is clear that four of the eight flames on either side are attached to the anterior branch of the collecting tube and four to the posterior. There are thus sixteen flame cells for which the Looss-Faust formula is $2\ (2 \times 4) = 16$.

Miracidium. Hickman described the miracidium of *Coitocaecum* from progenetic eggs. In its elongated swimming shape the larva is $0{\cdot}09 \times 0{\cdot}04$ mm. At rest, alive, it is $0{\cdot}08 \times 0{\cdot}05$ mm. Emergence from the egg may be either anterior or posterior end first; but in the latter case eclosion is muscular and not due to the action of the cilia. There is a blunt eversible trebratorium anteriorly, on which the apical glands open. A fairly large cell lies to each side of the trebratorium, and in the parenchyme there are twelve or more cells similar to formative cells (Fig. 6). Posteriorly there are two circular patches of refringent material which Hickman seems to regard as genital anlagen. Since each is located on a very large cell and agrees in form with the superficial structureless granules found in the sporocyst they probably are unconnected with the germ cells. A flame cell 0·005 mm. long is situated on either side of the miracidium, near the surface.

The animal swims rapidly, revolving about its long axis, shortening and extending periodically. Since there are no eye-spots there is no phototropism, in marked contrast with the miracidium of *Fasciola* or of *Opechona*.

Sporocyst. Potamopyrgus antipodum and *P. badia* both become infested with the parthenita of *Coitocaecum anaspidis*. A fully grown sporocyst reaches the size of $1{\cdot}35 \times 0{\cdot}30$ mm., and contains from thirty to forty-five cercariae, though in some cases only twelve may be present. Clearly the number varies with the age of the sporocyst and the number of cercariae previously released. The wall is thin, but faintly muscular at the germinal end (Fig. 7). Germ cells are localized in a narrower region posteriorly where there is an accumulation also of refractive amorphous material. At the opposite end there is a terminal birth pore which is probably a permanent structure, and not a result of the burrowing of the cercariae. The characteristic features of this sporocyst are then, the narrow germinal region, the refringent material scattered over the wall,

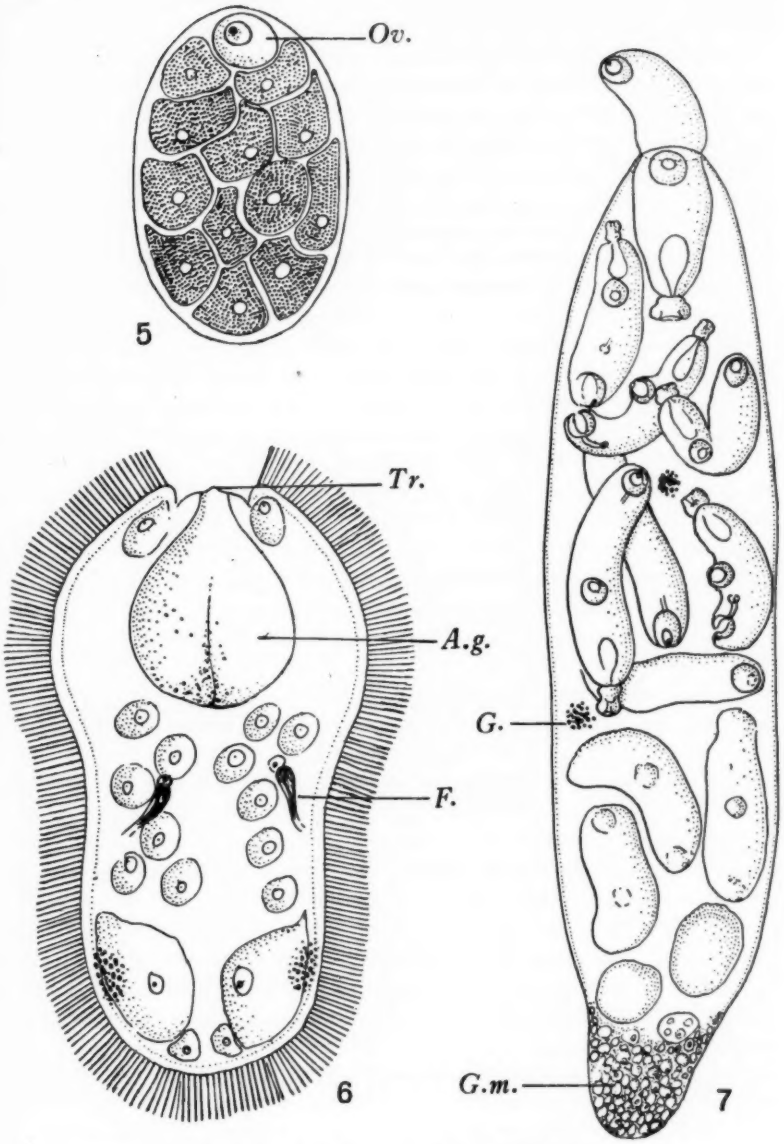

Fig. 5. Uterine egg of *Coitocaecum*. Size 0·047 × 0·077 mm.
Fig. 6. Miracidium of *Coitocaecum*. Size 0·08 × 0·05 mm.
Fig. 7. Sporocyst of *Coitocaecum*. Size 1·20 × 0·30 mm.

the terminal pore, and of course the nature of the cercariae being produced in the body cavity.

Cercaria. Mature sporocysts are filled with writhing cercariae of the cotylocercous form. The cuticle of the cercaria is without spines. Fully grown, the cercaria is 0·25–0·29 mm. long by 0·07–0·10 mm. at its widest part. The tail is stubby, 0·045–0·067 × 0·042–0·06 mm. in size (Fig. 8). An invagination of the distal end of the tail allows that organ to be used as a third sucker. Muscles attached to the deepest part of the invagination and terminal lips of the tail make the organ comparable in action with the suckers of the dibranchiate Cephalopods. The animal cannot swim in the manner of most cercariae but it is able to move rapidly over the substratum by means of the oral and caudal suckers.

The acetabulum is two-thirds of the body length from the anterior end. From this it follows that the neck region is long and mobile; a condition correlated with the method of locomotion. The acetabulum is a little larger than the oral sucker but not in the same proportion, as it is larger than the oral sucker of the adult. Both these organs have thin, incurved lips and regular crenulations on the sucking surface—due to the arrangement of circular muscles in bands. The dorsal side of the snout is wide and blunt; and the stylet (0·015 × 0·005 mm.) is set in a pouch above the oral sucker. On each side of the stylet are the openings of the penetration glands. The stylet is unusual in having a double instead of a single point. The spines are close together and 0·005 mm. long. Rudiments of the prepharynx and pharynx show clearly but nothing of the caeca appears.

Neutral red does not show the penetration glands readily, though usually the stain is definitive for those organs. But examination of the glands by reflected light with a black background showed their number and form. In these conditions the granules of secretion in the three glands on either side reflect light from their spherical surface, and are, with the turgid ducts, the only materials visible. The excretory vesicle is lined with irregular cells similar to those in the cercaria of *Telogaster* and *Opechona*. It is club-shaped, and extends from the acetabulum backwards to open near the base of the tail. One-fifth of the length of the vesicle from its anterior end the main collecting tubes enter. A slender posterior branch arises at acetabular level, while the thicker anterior tube continues forward to the penetration glands before it narrows in diameter. There are sixteen flame cells, each 0·007 mm. long, arranged in the pattern 2 (2 2 2 2). Genital rudiments have not been observed, though they are almost certainly segregated since they appear in early metacercariae.

Metacercaria. Specimens of *Paracalliope* over 2 mm. long are frequently infected with one, or sometimes two, cysts. Hickman, however, found up to twenty-four in an individual of *Anaspides*. The cysts lie in the haemocoele.

The identity of the cysts was not proved experimentally, but there is little doubt that they are the young stages of *Coitocaecum anaspidis* for the following

reasons: the smallest metacercariae retain the essential organs of the cercaria and the excretory systems are identical; amongst the cercariae found in *Potamopyrgus* spp. only the one described shows any formal agreement with the young metacercariae; there are no similar cercariae in the other molluscs of the bistopes concerned and none of these molluscs is sufficiently numerous to account for the observed extent of infestation of *Paracalliope*. In the Heathcote and Selwyn Rivers, where the cycle was studied, molluscs other than *Potamopyrgus* spp. formed about 0·1 % of the molluscan population.

Hickman has not touched on the metamorphosis, which occurs to a slightly marked extent. Trematode metamorphosis is better seen in *Telogaster opisthorchis*, to be described later. The process may be divided into four stages.

(*a*) Cysts 0·29 × 0·25 mm. have been found. The cercaria within these cysts is little more than bent ventrally (Fig. 12). This is due to the diaphanous nature of the primary cyst which is too delicate to retain spherical shape. Larval characteristics still present are the penetration gland ducts, the double-pointed stylet, 0·015 mm. long, the irregularly lined saccular excretory vesicle, and sometimes a trace of penetration gland (Fig. 13). No flame cells have been detected. In a specimen 0·36 mm. long, the larval organs mentioned were present, but the circular gut, the three gonad masses, and the adult shape of the acetabulum were present as well. The presence of gonads indicates that the development of the cercaria is often more advanced than it appears to be. Usually the pre-metamorphic stage of *Coitocaecum* completes the definition of the gut, so that metamorphosis concerns the genital ducts and parenchyme only.

(*b*) Granulation, vacuolization and fusion of the cytoplasmic elements occur in cysts about 0·3 mm. in length. The outline of every organ but the oral sucker, the pharynx and the acetabulum disappears.

(*c*) The post-metamorphic metacercaria has lost all larva organs. The gonads and their ducts are well defined; vesicula seminalis, cirrus and prostate glands have formed. Flame cells are visible again, arranged in the 2 × 4 × 2 pattern, while the walls of the excretory vesicle are very thin.

Growth takes place in the cyst, which, however, does not thicken appreciably. There is no more than a faint film contributed to the cyst by the host. With growth, the proportions of the body change. The neck region is relatively shorter; that is, the posterior region grows longer. This means a lengthening and narrowing of the excretory vesicle. The ratio of length of the metacercaria to the breadth changes from 1 : 4 to 1 : 3. The fourth great change of proportion, from the proportions of the larva, is that of the acetabulum, which in the cercaria was 0·056 mm., to the oral sucker's 0·050 mm. In the metacercaria the oral sucker is about 0·11 mm. in diameter; the acetabulum 0·21 mm., so that the ratio of the oral sucker (1) to the acetabulum is changed from 1 : 1 to 1 : 2. The characteristic wavy slit of the acetabular opening appears after the metamorphosis.

(*d*) Progenetic metacercariae are fairly commonly met with in *Paracalliope*,

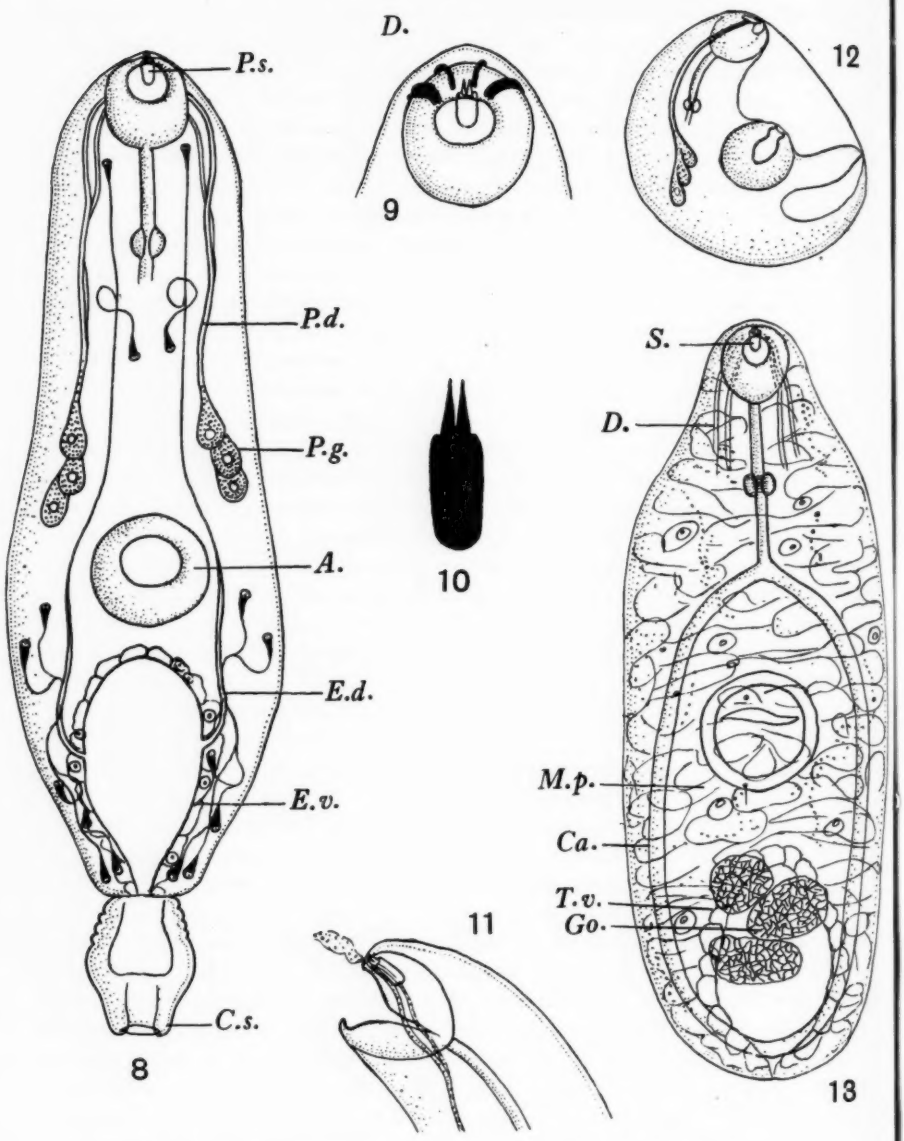

Fig. 8. Cercaria of *Coitocaecum*, ventral surface upwards. Size 0·27 × 0·09 mm.
Fig. 9. Penetration apparatus of cercaria from ventral surface.
Fig. 10. Stylet, 0·015 mm. long.
Fig. 11. Side view of anterior end of cercaria, to show penetration snout.
Fig. 12. Cercaria recently encysted in *Paracalliope*.
Fig. 13. Cercaria undergoing metamorphosis to the metacercaria of *Coitocaecum*.

though not so commonly as Hickman found them in *Anaspides* (200 progenetic out of 321 cysts). Usually they occur in the larger amphipods, 2·5–3 mm. long, and in these the cysts occupy all the space between the nerve cord and the heart. The cyst wall is thin, presumably because it lies in the haemacoele, and not in the solid host tissues where a secondary cyst would be accumulated.

Progenetic *Coitocaeca* are large, 1·5–2 mm. long by 0·48 mm. broad. The development of vitellarium and the gonads gives them a yellowish colour, and the parenchyma is more opaque than it is in the younger metacercariae. No sign of sperm has been seen in the testes, vesicula or receptaculum, but the ovary is filled with well-formed eggs. The vitelline follicles and the shell gland are fully functional, so that over 200 viable eggs may be produced. The undeveloped condition of the testes makes fertilization impossible so that the miracidia hatched are parthenogenetic, it seems.

Heavy infestation of tank bred *Gobiomorphus gobioides* has been produced by feeding cultures of infested *Paracalliope*. The life history of *Coitocaecum* is similar to that of *Hamacreadium mutabile*, a typical Allocreadiid, described by McCoy (1929). In the latter species the cercaria encysts in small fish, ready to mature in the schnapper. The cercaria is cotylocercous and nearly identical with *Coitocaecum* though it has four penetration glands and the stylet is single pointed. The adult is in some respects similar to the adult of *Coitocaecum*, but the caeca are not fused posteriorly. This is a case of larval similarity and adult deviation of the two genera. The life cycle and general anatomy of the Coitocaecinae show them to be true Allocreadiidae and indicate that the junction of the caeca posteriorly is a minor characteristic. It appears to be a more natural scheme that Poche (1926) should assign the circular gut forms only to a subfamily rather than to a family as Ozaki (1929) has done.

Paracalliope fluviatilis and the bionomics of *Coitocaecum anaspidis*

Paracalliope fluviatilis is the only New Zealand host that has been found for the metacercariae of *Coitocaecum anaspidis*. But besides the trematode, the cysticercoid of a hymenolepid cestode occurs in specimens of the amphipod from the Heathcote River, and the larva of a member of the genus *Acanthocephalus* in *Paracalliope* from the Selwyn River. The incidence of these larval helminths may be gauged from Table II. From 0·5 to 1·0 % of adult specimens of *Potamopyrgus* were infested with parthenita of *Coitocaecum*.

This crustacean lives amongst *Nitella* and other plants in mid-stream, and in the erosion cavities at the base of the banks. Experiment readily showed that it has a mild negative phototropism which keeps it in semi-dark places. A much stronger tropism is its positive thigmotactic reaction which keeps the animal on the sub- or suprastratum. It clings to any material available, from river shingle or *Nitella* to detritus and *Potamopyrgus*.

The cercaria of *Coitocaecum anaspidis* has no eyespots nor has it a definite phototropism. It has been noted already that its locomotion is strictly

terrestrial, since no swimming tail is present. The reactions of *Paracalliope* are such, then, that the cercaria may readily attack it. The thigmotropism of the host corresponds with the ground-living adaptation of the parasite. Not only do the *Potamopyrgus* species live among the aquatic plants with *Paracalliope* so that the cercaria may crawl from one host to another, but also the amphipod frequently settles upon *Potamopyrgus* and remains there for a number of minutes readily available for parasitization.

Table II. *Infection of* Paracalliope fluviatilis

Date	No. of specimens over 2·0 mm.	% *Coitocaecum* in specimens over 2·0 mm.	% hymenolepid cysticercoid in specimens over 2·0 mm.	% of each length group carrying *Coitocaecum*		
				2·5–3·0 mm.	2·0–2·5 mm.	1·5–2·0 mm.
April	29	27·0	—	62·5	14·4	0
May	32	25·0	6·5	46·0	6·0	0
June	104	14·7	9·0	32·0	10·5	0
August	189	5·1	2·0	—	—	—

The number of *Paracalliope* in which parasites were found is expressed as a percentage of the total number examined. In the length groups the number of parasitized amphipods is expressed as a percentage of the number of specimens in each group.

In the composition of Table II there are not sufficient numbers of specimens for detailed conclusions to be drawn. But the point emerges, that the parasite does not make its entrance into *Paracalliope* till that host has reached a certain size—a condition similar to that found in the parasitization of molluscs. Growth is rapid and breeding continuous in *P. fluviatilis*, so that there is a constant supply of animals of the requisite size. 2·0 mm. is the minimum length of animals found to be parasitized. If an amphipod shorter than 2·0 mm. were attacked it would no doubt die, since the cercaria is 0·30 mm. long and it quickly grows to 1·0 mm. Even the larger animals must be considerably disturbed by the presence of a progenetic cyst 0·8 mm. in diameter. From the table it is seen that virtually no parasites are found in specimens of *Paracalliope* shorter than 2·0 mm.; about one-tenth of the specimens between 2·0 and 2·5 mm. are parasitized; and that between one-third and one-half of those above 2·5 mm. in length carry *Coitocaecum*.

Small fish of the rivers (bullies, *Galaxias* spp., and eels) all use *Paracalliope* for food, and the *Coitocaeca* present are viable in all the host fish examined. Both the small fish and the amphipods spend much of their time amongst the plant growth at the edge of streams or in erosion cavities, so that *Paracalliope* is easily converted into food. The tendency of *Gobiomorphus* of 5 cm. and over to keep to the shadows results in their eating more of the negatively phototropic *Paracalliope* than the more phanerozoic young bullies. Larger bullies are thus more heavily infested with *Coitocaecum anaspidis*. But the cysticercoid has not been found to develop in any of the hosts mentioned. The *Acanthocephalus* larva develops in bullies, and is probably the same as the species found in the eel.

SUMMARY

The life cycle of *Coitocaecum anaspidis* involves three important stages. The adult occurs in *Galaxias brevipennis, G. attentuatus, Gobiomorphus gobioides,* and in specimens of *Anguilla* spp. less than 30 cm. long. *Potamopyrgus antipodum* and *P. badia* are the molluscan hosts of the sporocyst and cercaria. In New Zealand the cercaria attacks and encysts in *Paracalliope fluviatilis,* and in Australia in *Anaspides tasmaniae.* A metamorphosis of the cercarial larva into a progenetic metacercaria takes place in the amphipod.

Paracalliope does not become infected with *Coitocaecum* till the crustacean has reached 2·0 mm. in length. The thigmotropism of the *Paracalliope* fits exactly with the ground-living habit of the stumpy-tailed cercaria. The small river fish feeding in slack water acquire *Coitocaecum* from *Paracalliope.*

ACKNOWLEDGEMENT. I am glad to give my thanks to Prof. E. Percival of Canterbury University College for the kindly assistance and encouragement he has afforded me.

REFERENCES.

CHILTON, C. (1904). On the occurrence of a species of cercaria in the cockle. *Trans. N.Z. Inst.* **37**, 322–4.

HICKMAN, V. V. (1934). On *Coitocaecum anaspidis* (n.sp.), a trematode exhibiting progenesis in the freshwater crustacean *Anaspides tasmaniae* Thomson. *Parasitology,* **26**, 121–8.

HOPKIRK, C. S. M. (1927). The intermediate host of *Fasciola hepaticum* in New Zealand. *N.Z. J. Agric.* p. 175.

HUTTON, W. F. (1904). *Index Faunae Novae Zealandiae.* London.

LÜHE, M. (1909). *Trematoda. Die Süsswasser-fauna Deutschlands.* Jena.

MCCOY, O. R. (1929). The life history of a marine trematode *Hamacreadium mutabile* Linton, 1910. *Parasitology,* **21**, 220–5.

MACFARLANE, W. V. (1937). Liver fluke in New Zealand sheep. *J. Agric. N.Z.* **55**.

MONTICELLI, F. S. (1899). Notes on some entozoa in the collection of the British Museum. *Proc. zool. Soc. Lond.* pp. 321–5.

NICOLL, W. (1910). On the entozoa of fishes from the Firth of Clyde. *Parasitology,* **21**, 341–7.

—— (1915). Trematode parasites of Queensland. *Parasitology,* **8**, 22–41.

PIGULEWSKY, S. W. (1931). Neue Arten von Trematoden aus Fischen des Dnjeprbassins. *Zool. Anz.* **96**.

STUNKARD, W. H. (1930). An analysis of the methods of study of larval trematodes. *Parasitology,* **22**, 268–73.

YAMAGUTI, S. (1934). Helminth fauna of Japan. II. *Jap. J. Zool.* **5** (*Coitocaecum,* p. 356).

YOUNG, M. R. (1938). Helminth parasites of New Zealand. *Imp. Bur. agric. Parasit.* (Helminthology).

Key to lettering of figures

A.	acetabulum	*Oe.*	oesophagus
A.g.	apical glands	*O.s.*	oral sucker
C.	cirrus	*Ov.*	ovum
Ca.	caecum	*Ovd.*	oviduct
C.p.	cirrus pouch	*P.*	prostate
C.s.	caudal sucker	*P.d.*	penetration ducts
D.	ducts	*P.g.*	penetration glands
E.	egg	*Ph.*	pharynx
E.d.	excretory duct	*P.ph.*	prepharynx
E.p.	excretory pore	*P.s.*	penetration spine
E.v.	excretory vesicle	*S.*	spine
F.	flame cell	*T.*	testis
G.	granules	*Tr.*	trebatorium
G.m.	germ mass	*T.v.*	thick-walled vesicle
Go.	gonads	*U.*	uterus
G.p.	genital pore	*Va.d.*	vas deferens
L.	Laurer canal	*V.d.*	vitelline duct
M.	mouth	*V.f.*	vitelline follicle
M.p.	metamorphic parenchyma	*V.s.*	vesicula seminalis
O.	ovary	*Y.*	yolk reserve

(*MS. received for publication* 21. XII. 1938.—Ed.)

FACTORS AFFECTING THE MORPHOLOGY OF *LEISHMANIA TROPICA*

THE PRODUCTION OF *LEISHMANIA* FORMS IN CULTURES

By DAVID WEINMAN

From the Department of Comparative Pathology and Tropical Medicine, Schools of Medicine and Public Health, Harvard University

Introduction

Leishmania tropica, the parasite of Oriental Sore, was seen and certain aspects of its morphology recognizably described by Borovsky in 1898. Its appearance, as it is known to-day, was independently recorded in print and in photographs by Wright in 1903. Nicolle in 1908 showed that it assumed the flagellated form in cultures, as does the closely related kala-azar organism (Rogers, 1904). Henceforth both parasites were classified in the genus *Leishmania* Ross, 1904. Finally, Nicolle & Manceaux (1910) inoculated animals with cultures, and reproduced the essential features of the human lesion.

These findings have been confirmed repeatedly and Laveran's summary made in 1917 is a valid statement of our knowledge to date: "The Leishmania are found in two very distinct forms: the aflagellate form which is found in the tissues of man or of mammals infected with leishmaniosis; the flagellate form observed in culture of the preceding form" (translated).[1]

This dimorphism of all of the species of *Leishmania* is very striking, and its cause has aroused speculation. The factors effecting the change in form, however, have remained obscure. This obscurity resulted in large part from an inability to produce aflagellate forms under circumstances such that a variety of factors could be altered independently of one another. It is apparent that by the production of *Leishmania* forms *in vitro* such favourable circumstances would be provided.

The purpose of this paper is to show that it is now possible to obtain the aflagellate form of *L. tropica in vitro*, to detail the methods whereby this result has been obtained and to discuss some of the factors concerned in this process of morphological change.

Methods and material

The strain of *L. tropica* was isolated in Algeria and kindly furnished by Dr Marshall Hertig. It has been maintained in culture on N.N.N. medium for a number of years, without any animal passage for at least three years.

[1] An exception to this statement should be made for the extremely rare cases in which flagellate forms have been found in infected mammals.

In the present series of experiments, four different types of media were employed: (1) N.N.N. medium, (2) Noguchi's semi-solid serum agar, (3) tissue cultures explanted according to the technique utilized by Pinkerton (1936) and incubated at 32–33° C., and (4) pieces of tissue surviving on agar slants.

The substrate for the surviving cells in the fourth type of medium consisted of agar slants of which two types were employed. The more successful was the serum-Tyrode-agar devised by Zinsser *et al.* (1937). N.N.N. was less satisfactory. The serum-Tyrode-agar medium[1] was made as follows: Prepare and sterilize 150 c.c. of a 3 % suspension of agar. Melt the agar, cool to 50° C. and add the following mixture warmed to 30° C.: (*a*) 150 c.c. of sterile double strength Tyrode solution, plus (*b*) 100 c.c. of sterile horse serum, plus (*c*) 8 c.c. of a sterile 0·04 % aqueous solution of phenol red. All constituents of the medium are thoroughly mixed, the medium is then tubed, stoppered with cotton and slanted. After 48 hr. at 37° C. the cotton plugs are replaced with rubber stoppers, reincubated 24 hr. at 37° C. and stored at 8–10° C. Tubes three months old have given satisfactory results.

The tissues utilized were removed aseptically from guinea-pigs (killed under ether anaesthesia), and divided into pieces of about 2·5 mm. on a side. This size yielded good results, although larger and smaller pieces have also proved satisfactory. The tissue fragments were rapidly washed in Tyrode's solution, then bathed in N.N.N. fluid rich in *Leptomonas* forms for 15–30 min. and finally placed on the surface of the agar slant. About fifteen pieces of tissue were usually planted on agar slants contained in test-tubes measuring 15 × 1·3 cm. Two satisfactory kinds of tissue were employed, lung and spleen, of which the lung gave the better results. The following descriptions are based on the study of organisms and cells stained by the May-Grünwald Giemsa method.

Experiments and observations

When flagellated forms of *Leishmania tropica* were inoculated on to media containing surviving cells (type 4), after 4–13 days they assumed a variety of forms. The most prominent of these forms found in the midst of the tissue cells were typical leishmaniae presenting the appearance which they have in films from infected human tissues.

These *Leishmania* forms appeared ovoid or circular. The cytoplasm stained a light blue, it frequently contained chromatic granules and sometimes optically empty vacuoles. The nucleus was sharply defined and stained a clear bright red violet. A kinetoplast was always present, rhizoplasts were only rarely observed. *Leishmania* forms undergoing division were common. Either the nucleus or the kinetoplast may divide first to be followed apparently by division of the other and finally fission of the cytoplasm. On one occasion what appeared to be a single organism contained three nuclei.

The distribution of the aflagellate forms was very uneven and unequal. In

[1] This modified formula was kindly communicated to the author by Dr H. Wei.

a film of a single piece of tissue certain regions were rich, others poor in *Leishmania* forms. Similarly, in a single tube different pieces of tissue, which had received apparently identical treatment, showed different degrees of infection from a heavy one to none at all.

Flagellated forms were almost always present, as well as transitional ones intermediate between these and the *Leishmania*. The *Leptomonas* forms were particularly abundant in the drop of fluid surrounding the tissue. They were thus in less intimate contact with the cells than the *Leishmania* forms which were found interspersed among cells or contained in their cytoplasm. Intracellular forms were uncommon, and often appeared degenerated. Whether the extracellular forms ever resulted from extracellular multiplication is not known.

Some degenerated organisms were always found; they were most frequent after long periods of incubation when the cells also were showing evidence of degeneration. No detailed study of the effect of *L. tropica* on the cells was attempted, but the length of tissue cell survival was not notably different in cultures with abundant growth of *L. tropica* from those which remained sterile.

The medium sooner or later became acid, the indicator changing from red to yellow. At the time of change, which usually began after 5–10 days' incubation at 37° C., many of the cells and organisms had degenerated and shortly after, only degenerate forms of both were found. The period elapsing before acidity commenced varied inversely with the temperature. It was shorter with spleen than with lung, and varied to some extent with the individual guinea-pig from which the tissue was taken.

Some factors affecting the transformation were next studied. With the use of explanted tissue on N.N.N. medium abundant growth of both *Leishmania* and *Leptomonas* forms took place, although all tissue employed degenerated more quickly than when the serum-Tyrode-agar was employed.

The temperature of incubation was varied more than 10° C. with satisfactory results. Metamorphosis occurred at room temperature (22–26° C.), at 33 and at 37° C.; it was most extensive at 37° C.

The successful results attendant upon the use of guinea-pig tissue on agar slants made it conceivable that the susceptibility of an animal to leishmaniosis bore very little relation to the ability of its explanted cells to provide favourable circumstances for the production and multiplication of *Leishmania* forms. This was found to be the case, for tissue producing the metamorphosis of *L. tropica* was obtained from an animal far removed from all those subject to leishmaniosis: the frog. Splenic tissue of *Rana pipiens* placed on serum-Tyrode-agar slants, transformed the *Leptomonas* forms of *Leishmania tropica* into *Leishmania* forms, after 6–11 days' incubation at room temperature or at 33° C. The tissue was very extensively parasitized, and numerous *Leishmania* forms in division were encountered. The change in form took place sooner at 33° C. than at room temperature. On the whole, the results obtained were not so satisfactory as with the guinea-pig tissue.

The killing of the cells by heat had a profound effect on the growth of the flagellate. Guinea-pig lung or splenic tissue placed in Tyrode's solution and heated to 70° C. for 1 hr., no longer permitted transformation of *L. tropica*. The tissue cells appeared greatly altered in films; showed pycnotic black purple staining nuclei and were often without definite cytoplasmic outlines. Such heated tissue, bathed in N.N.N. fluid rich in *Leptomonas* forms of *L. tropica*, placed on serum-Tyrode-agar, incubated at 37° C. and examined 5–10 days later showed very marked degeneration of both cells and Protozoa. Thus, the conditions which permit *L. tropica* to assume the aflagellate form and to live and multiply at 37° C. are not afforded by such heat-killed tissue.

True tissue cultures were also decidedly unfavourable to *L. tropica*, and in thirty to forty such cultures neither form of the organism was obtained. Identical results were obtained when tissue growth was good, fair or non-existent. We have previously shown that explanted cells on agar slants were favourable to the development of *L. tropica*. Since the standard of type of tissue cultures inhibited this growth, the inhibiting factor or factors were first sought in the tissue culture medium. This medium consisted of a solid plasma clot or "bed" in which the infected tissue was embedded, and was made by mixing equal parts of guinea-pig plasma and guinea-pig splenic extract in Tyrode's solution. The bed without tissue was inoculated with *Leptomonas* forms of *L. tropica* and placed at 33° C., the same temperature at which the tissue cultures had been incubated. Forty-two cultures were each made by adding to one drop of a rich N.N.N. culture, one drop of splenic extract, mixing, and coagulating the whole with a drop of plasma; six additional cultures were made without the splenic extract. Six of these cultures, made with splenic extract and incubated at 37° C. became sterile. Forty-two other cultures were all incubated at 33° C.; all remained sterile except two. These last showed an abundant growth of *Leptomonas* forms only. Thus, in the tissue bed some factor habitually inhibited development of *Leishmania tropica*. Of the three components of the bed, the Tyrode's solutions and splenic extract mixture appeared to be innocuous, while plasma was definitely lethal for the organism. Accordingly, when N.N.N. fluid containing *L. tropica* was mixed with equal quantities of heparinized guinea-pig plasma, all of the *Leptomonas* forms became inactive in 15–35 min. after mixture. The immobility of the *Leptomonas* forms was not due to the rigidity of the plasma clot, for organisms in the exuded serum were also stationary. After 24 hr. at room temperature, the organisms so treated were still motionless and appeared to be undergoing degeneration. Plasma conserved in the refrigerator for 1 month at 5–8° C. exerted a similar effect. Splenic extract and Tyrode's solution mixed, when employed in similar fashion, exerted no like effect.

A set of experiments was devised to determine whether substances capable of affecting the *Leptomonas* forms would diffuse through the plasma clot. Tissues were embedded as usual, and after solidification of the plasma, each clot was covered by a drop of N.N.N. culture containing flagellates and this drop then

covered wholly or in part with a drop of plasma. The twelve cultures (six of lung and six of spleen) so made were incubated at 33° C., and after 4 days in three of them an abundant growth of *Leptomonas* forms was observed. Thus, *Leptomonas* forms placed in mediate contact with tissue cells through the interposition of a plasma clot were usually killed; when they survived no transformation took place.

DISCUSSION

Rhoda Erdmann (1915) was one of the earliest investigators to employ tissue cultures for studies of the Trypanosomidae. In 1934 Yen & Chung (1934) applied similar methods to organisms of the genus *Leishmania*. These authors utilized chicken embryo tissue which they infected with *L. donovani* cultures on N.N.N. medium. They obtained at 20° C. an abundant growth of the *Leptomonas* forms, whereas at 37° C. no forms of *Leishmania donovani* were to be found. They did not report *Leishmania* forms in their tissue cultures.

The present results were obtained with the use of guinea-pig tissue. The guinea-pig is naturally refractory to leishmaniosis yet abundant growth and multiplication of *Leishmania* forms of *L. tropica* were obtained by the utilization of guinea-pig tissue on agar slants at a temperature of 37° C. The behaviour of *L. tropica* on agar slants containing living cells differs from that on cell-free medium in two respects. First, morphologically, *Leishmania* forms were present. Second, physiologically, the organisms survived for long intervals at 37° C.

The cells of two very dissimilar animals (guinea-pig and frog) caused the organism to change from the *Leptomonas* to the *Leishmania* form. It is an attractive and not unlikely hypothesis to suppose that the identical effects were produced by similar causes, and that when these causes are known they will prove to be common to the cells of the two animals, perhaps residing in the cells of most of the vertebrates.

No transformation or survival of *L. tropica* was observed in the tissue cultures in which guinea-pig tissue was inoculated and explanted in a plasma clot. This result was probably caused by a lethal factor found to be present in the plasma. This factor may be identical with the substance designated "immobilin" by Knowles (1920) and described by Archibald (1914) and Cornwall & La Frenais (1916). Immobilin is present in the serum of the guinea-pig and many other animals. Such sera mixed, in sufficient concentration, with the cultural forms of *L. donovani* render it motionless. We obtained similar results with N.N.N. fluid containing *L. tropica* and heparinized guinea-pig plasma. The immobilin of the plasma appears to explain the high percentage of cases of non-development of *Leptomonas* forms when N.N.N. culture fluid was mixed with plasma and splenic extract, as well as the failure of the organism to develop in standard tissue cultures.

The guinea-pig cells in tissue culture may also have exerted a lethal action upon the organism, although the high mortality due to action of the plasma

alone rendered impracticable any firm conclusion in the matter. It should be noted, however, that of two animals, both of whose sera contains immobilin, one may be susceptible to leishmaniosis (i.e. man) the other refractory (i.e. the guinea-pig). The natural immunity of the guinea-pig may in part be due to some cellular factor. Whether this be the case or not, it is a striking fact that cultures with tissue from this refractory animal placed under conditions favouring cellular multiplication show the same resistance to infection which is a characteristic of the living animal, while cultures with similar tissue placed under minimally or non-reproductive conditions no longer evinced this resistance. These results appear to suggest the lowering of the "natural" resistance of explanted tissue by the alteration of external factors, results similar to those which have long since been obtained *in vivo*.

In the development of *Leishmania tropica* in association with tissues no previously undescribed morphological forms have been observed. No evidence supporting a schizogonic type of division was found. In several hundred dividing organisms one division was encountered which was consistent with, though not a convincing demonstration of, a tripartite division. From all other division forms, two daughter individuals were in the process of formation.

The methods outlined in this paper may facilitate the study of the insect flagellates. For certain flagellates which do possess vertebrate hosts, it is now possible to obtain *in vitro* the form found in such hosts. This has been achieved for a *Trypanosoma* (*T. cruzi*, Kofoid *et al.* 1935) and now for *Leishmania tropica*. Probably, like results will follow the application of similar methods to the other species of *Leishmania*.

It is possible that the flagellates which are parasites of man can now be differentiated *in vitro* from those which possess only invertebrate hosts by the attentive study of their cultural requirements and growth forms on various culture media. An organism which parasites invertebrates only, *Leptomonas ctenocephali* has been shown by Tyzzer & Walker (1919) to degenerate on N.N.N. medium at 37° C. in a few hours time; *Leishmania infantum* survived at least 48 hr. incubation at 37° C. We have found that *L. tropica* will survive incubation on N.N.N. medium at 37°C. for at least a week if daily transfers are made, and 3–4 days without transfer.

Thus, with regard to survival on N.N.N. at 37° C., organisms of the genera *Leptomonas* and *Leishmania* differ markedly. *L. tropica* tolerates body temperature for long periods in a culture medium containing cells. If under similar conditions, those insect flagellates which are parasites of invertebrates only, degenerated rapidly, then cultivation of an undetermined insect flagellate on a medium containing cells would afford a means of determining its ability to survive and multiply at a temperature within the range of mammalian body temperatures as well perhaps as the developmental form which was favoured by the mammalian tissue.

CONCLUSIONS

1. Growth of *Leishmania tropica* with the production of typical *Leishmania* forms has been obtained *in vitro* in association with surviving cells.

2. The cell donor may be an animal naturally refractory to infection with *L. tropica*. All experiments here reported involved the use of cells from such refractory animals, which were zoologically as unrelated as the guinea-pig (*Cavia porcellus*) and the frog (*Rana pipiens*).

3. In guinea-pig tissue cultures *Leishmania tropica* was rapidly killed. Guinea-pig cells surviving on agar slants, however, permitted an abundant growth of *Leishmania* forms.

4. In association with living cells both flagellate and aflagellate forms have survived, without transfer, 11 days' incubation at 37° C.

5. Two effects of the living cells on the flagellate were distinguished: (*a*) a temperature effect which permitted of prolonged existence and multiplication at 37° C., and (*b*) a morphological effect which transformed the *Leptomonas* forms into *Leishmania* forms.

6. *Leishmania* forms developed and multiplied only in association with living cells.

7. *Leptomonas* forms of *Leishmania tropica* in association with dead guinea-pig cells did not metamorphose and at 37° C. the flagellates were rapidly destroyed.

SUMMARY

Leishmania forms of *L. tropica* were obtained in cultures. The methods and results are given and discussed.

REFERENCES

ARCHIBALD, R. I. (1914). A preliminary report on some further investigations on kala-azar in the Sudan. *J. R. Army med. Cps*, **23**, 479–95.

BOROVSKY, P. F. (1898). On Sart Sore. *Voenno-Medicinskij Žurnal* (November), 925–41. From a translation by C. A. Hoare (*q.v.*).

CORNWALL, J. W. & LA FRENAIS, H. M. (1916). A contribution to the study of kala-azar. *Ind. J. med. Res.* **3**, 698–724.

ERDMANN, R. (1915). The life cycle of *Trypanosoma brucei* in the rat and in rat plasma. *Proc. nat. Acad. Sci., Wash.*, **1**, 504–12.

HOARE, C. A. (1938). Early discoveries regarding the parasite of Oriental Sore. (With an English translation of the memoir by P. F. Borovsky: On Sart Sore, 1898.) *Trans. R. Soc. trop. Med. Hyg.* **32**, 67–92.

KNOWLES, R. (1920). The peripheral blood in kala-azar. *Ind. J. med. Res.* **8**, 162–76.

KOFOID, C. A., WOOD, F. D. & MCNEIL, E. (1935). The cycle of *Trypanosoma cruzi* in tissue culture of embryonic heart muscle. *Univ. Calif. Publ. Zool.* **41**, 23–4.

LAVERAN, A. (1917). *Leishmanioses*. 519 pp. Paris: Masson et Cie.

NICOLLE, C. (1908). Culture du parasite du bouton d'Orient. *C.R. Acad. Sci., Paris*, **146**, 842–3.

NICOLLE, C. & MANCEAUX, L. (1910). Recherches sur le bouton d'Orient. *Ann. Inst. Pasteur*, **24**, 673–720.

PINKERTON, H. (1936). The application of tissue culture to the morphological and biological study and classification of Rickettsiae. *Arch. Zellforsch.* **18**, 343–59.

ROGERS, L. (1904). Preliminary note on the development of Trypanosoma in culture of the Cunningham-Leishman-Donovan bodies of cachexial fever and kala-azar. *Lancet*, **2**, 215–16.

TYZZER, E. E. & WALKER, E. L. (1919). A comparative study of *Leishmania infantum* of infantile kala-azar and *Leptomonas* (*Herpetomonas*) *ctenocephali* parasitic in the gut of the dog flea. *J. med. Res.* **40**, 129–76.

WRIGHT, J. H. (1903). Protozoa in a case of tropical ulcer "Delhi sore". *J. med. Res.* **5**, 479–82.

YEN, A. C. H. & CHUNG, H. L. (1934). Cultivation of *Leishmania donovani* in media of embryonic chick tissues. *Proc. Soc. exp. Biol., N.Y.*, **31**, 1258–9.

ZINSSER, H., WEI, H. & FITZPATRICK, F. (1937). Agar slant tissue cultures of typhus Rickettsiae (both types). *Proc. Soc. exp. Biol., N.Y.*, **37**, 604–6.

ADDENDUM

In a very recent paper Gavrilov and Laurencin (1938) state[1] they obtained growth of *Leishmania* forms of *Leishmania* (sp?) in tissue cultures composed of embryonic hamster liver, embryonic hamster extract and hamster plasma; chicken and guinea-pig tissue cultures did not give them satisfactory results.

[1] Gavrilov, W. & Laurencin, S., Application d'une méthode de culture de tissus à l'étude des Protozoaires, *Ann. Soc. Belge Méd. Trop.* **18**, 41–56 (1938).

(*MS. received for publication* 28. XII. 1938.—Ed.)

FERTILIZATION AND EGG PRODUCTION IN THE BED-BUG, *CIMEX LECTULARIUS* L.

By KENNETH MELLANBY[1]
The University of Sheffield

CONTENTS

	PAGE
Introduction	193
Fertilization and metabolic rate	194
Hormonal control of ovarian development	195
Utilization of the sperm	196
Summary	198
References	198

Introduction

Several accounts have been given of the curious method by which the female bed-bug is fertilized (Cragg, 1920; Christophers & Cragg, 1922). The male inserts his "penis" into the opening of the organ of Ribaga, which is a cleft in the right side of the 5th (apparent 4th) abdominal sternum. A mass of sperm injected enters the organ of Berlese, which is a circular mass of cells inside the abdomen adherent to the body-wall in the region of Ribaga's organ. There appears to be no duct leading from the organ of Berlese to the female reproductive tissues, and the sperm make their way through the haemocoele to the oviducts and ovaries. Many of them are to be found inside the spermathecae, which are pouches on either side of the common oviduct in the region where it is joined by the two lateral oviducts. Various workers have observed that a large volume of sperm may be injected but there is some disagreement as to its fate. Cragg (1923) gave an accurate account of egg production in *Cimex*. He found that there was no ovarian development in the virgin female, and that one fertilization would allow the production of as many as 173 normal eggs. Towards the end of the period of egg production, apparently when the sperm were almost exhausted, sterile eggs were produced. Fertilization by a starved male, which presumably gave a smaller volume of sperm, caused females to produce fewer eggs than did fertilization by a well-nourished male. When males and females were kept together, copulation occurred frequently, and provided that the females had sufficient food they laid normal eggs until they died. Abraham (1934) studied particularly the fate of the sperm; he considered that a large amount was necessary for successful egg production, not only to fertilize the eggs, but also to nourish the female. The sperm is, he states, absorbed by the cells of Berlese's organ and by all parts of the internal genitalia, and this

[1] Sorby Research Fellow of the Royal Society.

absorption is considered necessary for proper ovarian development. I find it difficult to agree with all of Abraham's conclusions, but it seems probable that the sperm plays some other part in the female besides the ordinary fertilization of the egg.

Fertilization and metabolic rate

There is no ovarian development in the virgin female. If a virgin female is fed, the blood is digested much less rapidly than in one that has been fertilized. Dissections show that in bugs kept at 30° C. the gut of a virgin 7 days after a meal is about as full as that of a fertilized female after 2 days. In the fertilized female much of the nourishment goes in egg production, but some must be exhausted because of the greater rate of metabolism. It is unsatisfactory to compare the longevity of fed bugs because of the production of eggs and its effects, but if unfed females, which do not produce eggs, are starved, some after fertilization and some still virgin, striking results are obtained.

Newly emerged adult females were placed separately in glass tubes containing pieces of blotting paper on which to rest, at 30° C. and 50 % relative humidity. Half of the females were fertilized. Care was taken to avoid disturbing the bugs during the whole experiment, which accounts for the great longevity (Mellanby, 1938). The fertilized bugs survived an average of 82·1 days (s.e. 2·1) and the virgins survived 134·0 days (s.e. 3·3). Thus, though no eggs were produced, the rate of metabolism of the fertilized bugs was so increased that they exhausted their reserves in two-thirds of the time taken by the unfertilized females. This experiment incidentally gives no support to Cragg's suggestion that the sperm may serve the female as an extra food reserve during starvation.

When a female has exhausted the sperm from one copulation, the general rate of metabolism, measured by the speed with which a blood-meal is digested, returns to that of a virgin.

As soon as a fertilized female is fed, ovarian development begins. There is a "latent" period before the eggs are ready to be laid and this period is shorter at higher than at lower temperatures. If a virgin female is fed and then fertilized several days after feeding, the eggs do not begin to develop until about 24 hr. later, by which time the sperm has migrated from the organ of Berlese to the spermathecae. There is then a further "latent" period before oviposition begins; the length of this period is the same as that which occurs in a fertile female after feeding. During the whole period before the eggs are laid the bug will have been using up the blood in her gut, and so the longer the time which elapses between feeding and fertilization the fewer the eggs in that batch. At 30° C. some bugs fed after fertilization produced on an average 10·0 eggs, while those fertilized 8 days after feeding, by which time the others had finished laying, produced only 2·7 eggs. The fact that the latter group were able to produce any eggs at all such a long time after a meal shows how low the rate of metabolism of the virgin must be.

HORMONAL CONTROL OF OVARIAN DEVELOPMENT

Wigglesworth (1936) has shown that in the bug *Rhodnius* ovarian development is probably dependent on the secretion of a special hormone produced by the *corpus allatum*. The latter is stimulated to secrete when the female takes a blood-meal. He has also shown that moulting and "metamorphosis" are controlled by hormones in both *Rhodnius* and *Cimex*, and that the hormone controlling these processes in one species can induce the same changes in the other. We might therefore expect that in *Cimex* the control of egg production would resemble that in *Rhodnius*. The most noteworthy difference between the behaviour of the two species is the production of sterile eggs by virgin *Rhodnius* whilst there is no egg production in virgin bed-bugs. But once *Cimex* has been fertilized it appears to act in a manner similar to *Rhodnius*.

When a fertilized bed-bug is fed, there is first a "latent" period of about 5 days (at 24° C.) and then a batch of approximately a dozen eggs is produced at the rate of three per day. A ligature behind the prothorax cuts off the *corpus allatum* from the rest of the body (Wigglesworth, 1936); the abdomen and hind part of the thorax may then survive for many days. If a ligature is made within 3 hr. of feeding no eggs develop, and the blood is digested at the rate observed in virgins. A ligature made 24 hr. after feeding does not affect egg production. None are actually laid, but dissection 6 days after feeding may reveal as many as 12 fully developed eggs, most of which will hatch in a normal manner. This experiment in conjunction with Wigglesworth's work on *Rhodnius* indicates that feeding a fertilized bed-bug stimulates the *corpus allatum* to produce the hormone which in turn causes proper ovarian development. Insufficient hormone is produced in the first 3 hr. after feeding, but after 24 hr. no further secretion is needed by the ovaries.

I have been unable to observe ovarian development in any female *Cimex* which has not been fertilized in the natural manner. Abraham (1934) injected sperm into the haemocoele and obtained no eggs. I repeated his experiment with the same result. I then made numerous experiments in which I transplanted the following tissues into haemocoeles of recently fed virgin *Cimex* females:

(1) Organ of Berlese still filled with sperm from newly fertilized females.
(2) Spermathecae filled with sperm from recently fertilized females.
(3) Vesiculae seminales distended with sperm from well-nourished males.

None of these implantations appeared to have the slightest effect on the ovaries. In none of the implantation experiments did the sperm migrate to the spermathecae as they do after copulation. It appears then that the ovary only develops when the sperm have reached that region. Stimulation cannot be due to some substance in the blood stream liberated either by the sperm, or by the organ of Berlese after fertilization. Sperm must reach the correct part of the female system; after that the ovarian development is controlled by the hormone from the *corpus allatum* in the same way as in *Rhodnius*.

Utilization of the sperm

Dissections show that masses of living sperm can always be found in the spermathecae of females which are laying fertile eggs. Some weeks after fertilization sterile eggs may be produced. This seems to happen when the sperm are exhausted, for none are then found in the spermathecae. It appears that the influence of the sperm on the development of the ovary persists for a short time after the sperm themselves have disappeared, but usually after a few sterile eggs have been produced the ovary and the general rate of metabolism returns to the "virgin" condition.

Abraham (1934) considers that the main function of the spermathecae is to absorb the substance of the sperm and he calls them "*Resorptions-organen*". I cannot accept this conclusion, for masses of living sperm are found in the spermathecae for several weeks after copulation. The spermathecae may not be morphologically homologous with those of other insects, they have for instance no chitinous lining, but they undoubtedly preserve the sperm alive in the usual manner.

The sterile eggs have been described as "imperfect" or "malformed", but they are actually quite normal except for not being fertilized. If they are examined in a dissected female, or immediately after being laid, or even some time after being laid in saturated air, they will be seen to be of normal proportions. Any shortage of sperm has not affected the capacity of the female to produce eggs with their full complement of reserves. In dry air the eggs soon shrivel up, as the chorion gives insufficient protection against desiccation; it is evidently the properties of the serosa which enable fertilized eggs to withstand dry conditions.

The following experiments were made to find out whether the exhaustion of sperm in a female takes place after a definite number of eggs has been produced, or after a definite length of time. Cragg (1923) found that when a well-nourished male had fertilized a female, she produced more eggs than after copulation with a starved male. It is thus necessary to compare the effects of other conditions on females fertilized by males in the same physiological condition. I found it impracticable to use females known to have copulated once only. In each experiment I used females all of which had been kept with similar males for the same length of time. Dissections showed that under these conditions approximately the same amount of sperm appeared in the spermathecae.

If fertilized female bugs are fed at infrequent intervals, they produce batches of eggs following each meal, but if they are fed frequently they produce an almost continuous series of eggs. Thus in a given time, within limits, the more frequently she is fed the more eggs a female will produce (K. Mellanby, 1939).

Virgin females were placed separately in 3 × 1 in. glass tubes in an incubator at 24° C., and a newly fed male was placed in each tube for 2 days. Each tube

was covered with gauze and contained a piece of blotting paper, on which eggs were laid. Half the females were offered food every 4 days, and the remainder only a third as often. The results are given in Table I. It will be seen that though the frequently fed bugs produced more than twice as many eggs as the others, they did not exhaust their sperm any more rapidly. This suggests that the sperm can only survive for a definite and limited period in the female, and that the number of eggs produced has little effect on the exhaustion of the sperm.

Table I. *Production of eggs by female bed-bugs fed at frequent and infrequent intervals.* 24° C.

	No. of fertile eggs	Period during which fertile eggs were laid days	No. of sterile eggs	Period during which sterile eggs were laid days
Fed frequently	99·4	68·4	22·4	20·3
Fed seldom	43·8	64·2	7·6	14·4

As fertilization with only a meagre amount of sperm gives rise to few eggs (Cragg, 1923), it was thought that fertilization with really large quantities of sperm might last for a very long period. Virgin females were first put each with about a dozen recently fed males. The females were repeatedly fertilized, and dissections showed immense quantities of sperm inside their bodies. It appeared, however, that 12 males were too many, as some of the females died.

Table II. *Production of eggs by female bed-bugs fertilized by one or many males.* 30° C.

	No. of fertile eggs	Period during which fertile eggs were laid days	No. of sterile eggs	Period during which sterile eggs were laid days
Fertilized by many males	64	50	22	18
Fertilized by one male:				
(*a*) Fully fed once per week	58	48	20	8
(*b*) Only partially fed once per week	23	52	4	16

When six males were placed with one female in tubes for 2 days the females all survived, and were found on dissection to contain a great deal more sperm than other females kept with only a single male for the same period. In this experiment the bugs were all kept at 30° C. and 80 % relative humidity. They were fed once a week, but of those fertilized by one male half were removed from the rabbit on which they were feeding when they had only taken partial feeds. It will be seen from Table II that the sperm in all cases lasted approximately the same period, any differences not being statistically significant, and that the massive quantities of sperm gave no more eggs than did fertilization by a single male. Partial feeding, like infrequent feeding, gave few eggs but did not cause the sperm to last any longer. It will be noted that at 30° C. the sperm lasted for less time than it did at 24° C. The period for which it lasts seems to bear little

relation to the amount inserted at copulation, provided more than a minimum quantity, which is evidently more than a starved male can produce, reaches the female. Comparing my results with those of Cragg it appears that a single copulation with a well-nourished male is as efficacious as frequent fertilizations by several males, provided of course that these frequent fertilizations all occur at about the same date.

When a number of fertilized females were kept at 8–10° C. for a month, ovarian development was inhibited, but when these females were put at 30° C. they continued to produce eggs for a month longer than did controls kept at 30° C. for the whole period.

During the period before the sperm from one copulation was exhausted, females produced as many eggs as did others which were fertilized all through the experiment at frequent intervals, provided that both lots fed equally often. Thus the extra sperm again gave no evidence of possessing nutritive qualities.

Summary

Previous workers have described the curious method by which the female bed-bug is fertilized. They have suggested that the large quantity of sperm injected serves some function other than that of fertilizing the egg.

The rate of metabolism of the virgin bug is much less than that of the fertilized female.

Ovarian development never occurs until the female has been fertilized. After fertilization, egg development is apparently controlled by a hormone produced by the *corpus allatum*.

Living sperm will always be found in the spermathecae of females which are laying fertile eggs.

The so-called imperfect eggs produced when the sperm is exhausted are normal save for being unfertilized. They have been considered as malformed because of their tendency to shrivel quickly in dry air.

Sperm remains viable in the female for a definite period depending on the temperature. This period is longer at low than at high temperature. Provided a certain minimum amount of sperm is introduced (this minimum is less than the quantity introduced by a well-nourished male at one copulation), the period of viability of sperm is the same no matter how many eggs are produced. The number of eggs depends only on the amount of food taken.

There is no evidence that the sperm serves any nutritive function in the female.

REFERENCES

Abraham, R. (1934). Das Verhalten der Spermien in der weiblichen Bettwanze (*Cimex lectularius* L.) und der Verbleib der überschüssigen Spermamasse. *Z. Parasitenk.* **6**, 559–91.

Christophers, S. R. & Cragg, F. W. (1922). On the so-called "penis" of the bed-bug (*Cimex lectularius* L.) and on the homologies generally of the male and female genitalia of this insect. *Indian J. med. Res.* **9**, 445–63.

CRAGG, F. W. (1920). Further observations on the reproductive system of *Cimex*, with special reference to the behaviour of the spermatozoa. *Indian J. med. Res.* **8**, 32–79.

—— (1923). Observations on the bionomics of the bed-bug, *Cimex lectularius* L., with special reference to the relations of the sexes. *Indian J. med. Res.* **11**, 449–73.

MELLANBY, K. (1938). Activity and insect survival. *Nature, Lond.*, **141**, 554.

—— (1939). The physiology and activity of the bed-bug (*Cimex lectularius* L.) in a natural infestation. *Parasitology*.

WIGGLESWORTH, V. B. (1936). The function of the *corpus allatum* in the growth and reproduction of *Rhodnius prolixus* (Hemiptera). *Quart. J. micr. Sci.* **79**, 91–121.

(*MS. received for publication* 11. I. 1939.—Ed.)

THE PHYSIOLOGY AND ACTIVITY OF THE BED-BUG (*CIMEX LECTULARIUS* L.) IN A NATURAL INFESTATION

By KENNETH MELLANBY[1]

The University of Sheffield

(With 3 Figures in the Text)

CONTENTS

	PAGE
Introduction	200
Technique	201
Analysis of captures	202
Periodic activity	207
Population density and longevity	209
Summary	211
References	211

Introduction

During recent years the physiology of the bed-bug has been studied extensively in the laboratory, but much less work, except for empirical experiments on fumigation, has been done on wild populations under natural conditions. Opportunity to study the bed-bug under natural conditions was afforded by the discovery of an infestation in an animal room used for keeping and breeding rats. In spite of the fact that the rats were being used for other purposes and there was no control over their numbers, more freedom for study was available than would have been the case in a human habitation.

The room measured approximately 4 by 3½ m. and was 3½ m. high. There was a row of windows about 2 m. above the floor along one of the longer walls. The windows were double and did not admit direct sunlight. Iron racks which bore the rat cages covered three walls up to the level of the windows. The room was heated by steam pipes, and in winter the temperature seldom fell below 20° C. (68° F.) and seldom rose above 27° C. (80·6° F.). In summer the heating was sometimes turned off and in cool weather the temperature occasionally fell below 20° C. The floor was of concrete and the walls of smooth plaster, but cracks and particularly the spaces surrounding the pipes in the walls afforded shelter to the insects. Most of the bugs, however, seemed to lurk in the crevices in the metal cages containing the rats, and these animals seemed to form the only food supply for the bugs. Johnson's (1937) results on feeding these insects on various species of host suggest that their metabolism will not differ widely from that of others fed on man. The insect population fluctuated in numbers; when there was a large number of rats inhabiting many cages, the

[1] Sorby Research Fellow of the Royal Society.

bugs became more numerous, particularly as there was then less time for cleaning. When the stock of rats was rearranged some of the cages were usually cleaned and any bugs in them killed. Usually during the summer vacation the stock of rats was reduced, and all surplus cages were cleaned. This reduced the number of bugs considerably. Occasionally special efforts have been made to exterminate the bed-bugs, but some survivors have always escaped and the numbers have then increased to the previous level. This rapid increase in numbers shows how favourable the general conditions must have been for the insects.

The only enemies other than man from which these bugs suffer are the rats on which the bugs feed. It is probable that the rats eat many of the bugs. I have found that if a number of hungry bugs are confined with a rat for a night, nearly all the bugs are eaten and very few feed and survive. In two such experiments, out of twenty-seven bugs only six were recovered. When two rats instead of one were used, nineteen out of thirty bugs survived. The two rats tended to curl up together and sleep, and the attacks of the insects may have passed unnoticed. In the animal room where several rats were kept together in each cage, the bug mortality must have been lower than in the experiments mentioned above, otherwise the insects would soon have been exterminated, but it is probable that many were killed by the rats. In an infested house the killing of bugs by the human inhabitants would have a similar effect on the insect population.

The animal house harboured many cockroaches as well as bed-bugs, but it is unlikely that the cockroaches attacked the bugs. The common belief that cockroaches may keep down bed-bugs seems to have little foundation in fact (Johnson & Mellanby, 1939).

TECHNIQUE

The little field work which has previously been carried out on the bed-bug has consisted of examinations of insects which have been discovered and removed from their hiding places. Useful as such results may sometimes be, they give little information about the normal activities of the insects.

I have been able to trap bed-bugs which have left their hiding places voluntarily during their periods of normal activity. Examinations of these insects tell us a good deal about their habits. Two kinds of traps have been used, "Demon" cockroach traps and rolls of corrugated paper. In each experiment four "Demon" traps were placed, each one always on the same spot on the floor of the rat room. The bugs in their wanderings climbed up the sides of the traps, fell in and appeared to be unable to climb out again. All stages of nymphs as well as adults were caught by this method. In the second method small rolls of corrugated paper were left on the floor of the room, each roll consisting of a cylinder of paper 10 cm. long and 4 cm. in diameter; the bugs found hiding places in the folds of the paper. Héraud (1916) suggested trapping bugs in

corrugated paper as a control method. The experiments described here show that quite different types of catches were obtained by the two methods.

Thermograph or thermohygrograph records were kept in the animal house during the whole period of the experiments.

Some bed-bugs were released again after being caught; these were marked in such a way that each individual might be easily recognized. The marking technique is essentially similar to that used by entomologists to mark tsetse flies (see Jackson, 1933). Small spots of ordinary oil paint were put onto each bug in the positions marked in Fig. 1. A fine pin makes a suitable "brush". If only one spot of paint is used, 25 different markings can be made; with two spots of the same colour there are 300 marks available. Using more than two spots and combinations of several colours, it is possible to make many thousands of easily recognizable marks. If two spots are used it is simplest to refer to the patterns in the following manner: if one spot is in position 1 and the other in position 9, that is represented by 1/9 in order to distinguish it from a bug with one spot in position 19.

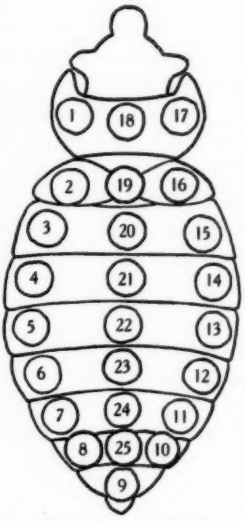

Fig. 1. Method of marking released bed-bugs.

Analysis of captures

The traps were generally left in the animal house at about 5.30 p.m. and removed at 9 a.m. when the insects were collected and examined. Table I shows briefly some of the results obtained. It will be seen that in March and May (1938) the "Demon" traps caught similar numbers of bugs, and there is no difference in the proportions of the various stages for the two months. During

Table I. *Details of bed-bugs captured*

	Fed bugs								Unfed bugs								
	Stages of nymphs					Adults		Total fed	Stages of nymphs					Adults		Total unfed	
Date	I	II	III	IV	V	♂	♀	bugs	I	II	III	IV	V	♂	♀	bugs	Total
	In "Demon" traps																
March, 14 days	58	39	40	19	33	7	17	213	143	128	105	76	54	107	80	693	906
May, 9 days	41	23	13	6	11	16	18	128	97	83	69	49	33	113	71	515	643
Aug.–Sept., 11 days	4	—	—	4	5	1	2	16	11	10	13	8	11	18	10	81	97
	In corrugated paper																
March, 13 days	34	39	37	18	30	8	7	163	8	7	4	6	1	7	3	36	199

March, collections were also made in the corrugated paper (Table I). The principal difference in the catches made by the two methods of trapping is that 77·9 % of the bugs caught in the "Demon" traps were unfed, whereas only

18·1 % of those found in the corrugated paper were unfed. The reason for this difference is easily seen. Once a bug was inside a "Demon" trap, it was unable to escape, but when a bug crawled inside a roll of corrugated paper it could as easily crawl out again. Hungry bugs seeking food would not remain in the paper, but fed individuals would find a snug hiding place. This suggests that trapping with corrugated paper would be unlikely to prove as successful for controlling bugs as Héraud (1916) thought. Incidentally a very moderate amount of cage-cleaning proved much more successful in reducing the numbers than any amount of trapping by either method. It is probable that the "Demon" traps caught such a small proportion of fed bugs because many newly gorged individuals, being less agile after having ingested a considerable weight of blood, tend to remain near the cages in which they have fed. Evidence given later indicates that practically all the unfed bugs captured in the "Demon" traps were hungry individuals which had left their hiding places to seek for food.

During July the rat population was reduced, and many of the cages were cleaned out. As a result the number of bugs was so greatly reduced that the average catch during August and September fell to approximately 9. When the heating was turned off during July, the temperature was sometimes lower than that maintained in the earlier part of the year, and thus breeding was probably also checked. This view is supported by the figures in Table I. In March and May small nymphs belonging to the first two instars made up 39 % of the total catch. In August and September only 26 % of the catch consisted of these young stages. However, the numbers caught during the later months are rather small, and too much importance should not be attributed to this result. During August and September the heating system was working once more, and so the small catches are due to a true reduction in numbers and not to any inactivity of the insects.

Many of the adult bugs captured were weighed, dissected and examined generally for their "hunger stage" and sexual condition. Of the males, 147 individuals selected at random from the hungry individuals caught in the "Demon" traps were found to have an average weight of 4·0 mg. (S.E. 0·09), and they ranged from a maximum of 6·5 mg. down to a minimum of only 1·5 mg. Sixty-two females were weighed, and their average weight was found to be 5·4 mg. (S.E. 0·17), the heaviest unfed female weighing 8·3 mg. and the lightest 3·1 mg.

It is possible to obtain some idea of how frequently wild bugs feed by comparing the weights of those captured with the weights of captive bugs kept for various periods after a meal under similar conditions of temperature and humidity. I do not consider that such a comparison by itself is particularly valuable, unless something is known about the amount of activity of both lots of insects. As I have previously shown, the activity of a bug has a considerable effect on its metabolism, and hungry individuals compelled to take even a moderate amount of exercise may live only a fraction of the time for which

inactive individuals survive (K. Mellanby, 1938). This effect of exercise seems to be most marked when the bugs have been subjected to starvation for several days, but even a few days after feeding 5 min. forced activity may have important consequences, as will be seen from the following experiments.

Some of the males caught in the traps were fed and then confined separately in glass tubes at 24° C. and 50 % relative humidity. At the end of 4 days the average weight of twenty-three individuals which were left undisturbed was 67·2 % (S.E. 1·52) of their weight immediately after feeding. Sixteen similar bugs were treated in the same manner, except that on the 3rd day after feeding they were kept running for 5 min. on an illuminated surface of blotting paper. On the 4th day their average weight was only 58·5 % (S.E. 3·11) of that after feeding. The different losses of weight in these two experiments are statistically significant. A similar experiment was made in which bugs were first weighed 4 days after feeding, some were exercised for 5 min. on the 7th day, and the weights on the 8th day were compared. Expressed as percentages of the weights on the 4th day, that of the resting bugs had fallen to 83·8 % (S.E. 2·0), and of those which were exercised, to 77·4 % (S.E. 1·5). The difference between these results is also statistically significant.

It is probably safe to assume that the male bugs caught seeking for food had taken an amount of exercise equivalent to at least 5 min. forced activity. The average weight of these wild bugs was 4·0 mg. The average weights of the exercised bugs 4 and 8 days after feeding were 5·1 and 3·4 mg. respectively. As both captive and wild bugs were living under approximately the same climatic conditions, these results suggest that the males in the animal house were feeding at intervals of between 4 and 8 days. Until more is known about the amount of activity of the wild bugs, this must only be taken as an approximate estimate. More accurate information may be obtained by examining the females captured.

With starved females activity seems to affect longevity in the same way as males, but during the period of egg production which follows soon after feeding, the rate of metabolism is so high already that extra activity does not seem to have much additional effect.

A comparison of the egg production of captive bugs and of trapped females is given in Fig. 2. The captive bugs were fed every 12 days. During the first 5 or 6 days after feeding the eggs develop, and then a batch of approximately twelve is laid at the rate of about three per day (Fig. 2, curve *A*). If captive bugs are fed every 12 days, they will continue to give results approximating to curve *A* for months, provided they are fertilized (see K. Mellanby, 1939). If, however, they are fed more frequently, about every 4 or 5 days, then they will lay an almost continuous series of eggs, producing as many as ninety in a month. Thus the more frequently the bug feeds, the more eggs she will produce; the cumulative effect of the frequency with which the females feed under natural conditions on the number of individuals produced in several generations will be enormous. The following experiments were made to find out whether the

wild females do not feed again until the eggs produced from the substance of the first meal have all been laid, or whether they feed more frequently. A bug which feeds every 5 days must of necessity feed a second time before the eggs, produced as a result of the first meal, are laid. These eggs are laid as the second meal is being digested.

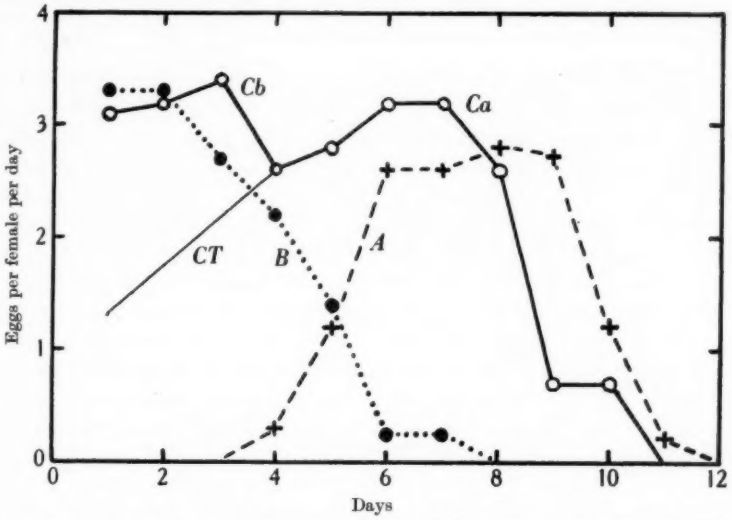

Fig. 2. Egg production by female bed-bugs. Curve *A* (broken line) = captive bugs fed at 12-day intervals—last fed on day 0. Curve *B* (dotted line) = wild bugs captured on day 0, unfed. Curve *C* (heavy line) = wild bugs captured and fed on day 0. For further description see text.

Some of the unfed female bugs captured in the traps were confined separately under conditions of temperature similar to those occurring in the animal house. Many of these bugs produced eggs during the first 24 hr. Curve *B* in Fig. 2 depicts the egg production of such females. On the assumption that they had fed 5 or 6 days before trapping, it will be seen that the results approximate to those obtained with the captive bugs (curve *A*). Another selection of the females captured in the animal house were allowed to feed and again many of these produced eggs during the first 24 hr.; their subsequent egg production is shown in curve *C*. Curve *C* is obviously approximately the sum of curves *B* and *A*. We may assume that the eggs represented by the first part of the curve, *Cb*, were produced from a meal taken several days before capture. The remaining part of the curve, *Ca*, represents the eggs produced from the meal given after capture.

All the females do not appear to feed quite so frequently. Table II shows the period elapsing between trapping and egg production; the results are compared with those obtained using captive bugs fed at infrequent intervals (cf. Fig. 2, curve *A*). It will be seen that nearly 60 % of the wild bugs laid eggs

Table II. *The number of days after feeding on which individual female bugs started to lay eggs*

	Days						
	1	2	3	4	5	6	7
Caught in trap, fed at once	31	11	10	6	11	4	1
	41·9 %	14·8 %	13·5 %	8·1 %	14·8 %	5·4 %	1·4 %
Captive bugs fed as soon as last egg in batch was laid	0	0	3	3	17	17	4
	—	—	6·8 %	6·8 %	38·6 %	38·6 %	9·1 %

during the first two days after capture, thus indicating that they were seeking for a meal before they had laid the eggs produced from the substance of the last one. Some of the remainder were feeding at less frequent intervals. This is shown by the line *CT* on Fig. 2 which is based on results obtained with a random and not a selected sample of trapped females. If all the females are considered instead of only those which start laying the day they are captured, the rate of egg production is indicated by the whole curve *CT–Ca*. Briefly this may be taken to mean that about 60 % of the female bugs were feeding at approximately 5-day intervals, and the rest, perhaps because they had been unlucky in their search for food, were feeding rather less frequently. Under natural conditions in the animal house most of the bugs would have fed again on about the 6th day, and so the rate of egg production would have remained up at the high level for several more days instead of falling steeply on the 10th and 11th days.

It was thought at first that some of the females caught in the traps might not have been searching for food but rather for places in which to lay eggs. Examination of those females which had actually fed before being caught showed this view to be incorrect, for both these bugs and the unfed individuals contained fully-developed eggs in their bodies in the same proportions.

The average weight of the unfed trapped females was 5·4 mg. (S.E. 0·17) while that of captive bugs the day after they had finished laying a batch of eggs was 4·15 mg. (S.E. 0·16); these had fed 10 days previously. These figures support the view that the wild bugs were feeding at intervals of considerably less than 10 days.

Cragg (1923) showed that *Cimex* females could be successfully fertilized very soon after their last moult and even before they had fed. He also states as a result of laboratory experiments that he believes that one copulation in 24 hr. is a minimal estimate in a wild population. I consider that this is an exaggeration. I dissected many of the female bugs captured in the animal house and most of them contained less sperm than did females from mixed laboratory cultures. Comparison with laboratory bugs whose past history was known indicated that the majority of the wild bugs had not copulated during the last week. The sperm in a fertile female remains in the spermathecae for several weeks after fertilization (K. Mellanby, 1939). The more frequently the female has been fertilized the more sperm there is apparent. It is difficult to make an accurate numerical comparison of the amounts of sperm in two females, but I devised a rough arbitrary scale in which a distended spermatheca

was represented by 6, an empty one by 0, and intermediate distensions by the numbers 1–5. A female with two well-filled spermathecae would be represented by 12. I dissected a number of females from the animal house, and compared them with females from a laboratory culture. In order that it should be a true comparison and that the standard should not alter I dissected alternately wild and laboratory bugs. The average figures for the amount of sperm were, laboratory bugs, 10·2, wild bugs 4·3. I also found that just over 10 % of the wild females were not fertilized; some of them had fed since their last moult, so they had not been fertilized for several days. Another fact which supports the view that the females were not fertilized at frequent intervals is the production of sterile eggs by some of those captured in the traps. This only occurs with bugs several weeks after their last copulation when the sperm is exhausted. These various findings indicate that the results of laboratory experiments, particularly those dealing with the behaviour of insects, must be treated with considerable caution. The sexual habits of wild bugs and of those kept in laboratory cultures are by no means the same.

PERIODIC ACTIVITY

In infested houses, bugs are normally active mainly at night. It is commonly believed that a light left burning will inhibit their activity. The experiments described in this section were made to investigate the periodicity of the bed-bug's activities.

"Demon" traps were used to capture the insects and the traps were examined at 3-hourly intervals throughout the night. The removal, emptying and replacing of the traps at the end of each 3 hr. period were carried out in 5 min., and without switching on the light. Results obtained on four nights during May 1938 are summarized in Fig. 3. The maximum catch was obtained at the same time during each night. Each point on the graph indicates the number of bugs collected during a period of 3 hr., and the times marked on the abscissa indicate the middle of each period. Thus 0130 represents the collection made between midnight and 3 a.m.[1] It appears that the bugs were most active after 3 a.m. During most of this period the room was not dark, for the sun rose shortly after 4 a.m.

Since it was apparent that the activity of the bugs was not simply correlated with light and darkness, attempts were made to correlate it with other factors. Changes in temperature were certainly not responsible for the periodicity, for it was noticed that on two nights when collections were made, in one case the temperature rose steadily throughout the night and on the other it fell, yet the bugs appeared at exactly the same times. There also appeared to be no correlation with changes of atmospheric humidity.

It is obviously desirable to make collections at other times of year when the sun rises at a later hour. Several experiments were performed in September, when the hours of sunrise and sunset are quite different from those in May.

[1] British summer time is used here. Thus "midnight" is 1 hr. earlier than true midnight, Greenwich Mean Time.

Unfortunately the bug population was then so few that the results and numbers obtained were insufficient to allow a definite conclusion to be drawn, but it was obvious that the periodicity of the activity was not greatly different from that found in May. Since September, special measures against the bugs have pre-

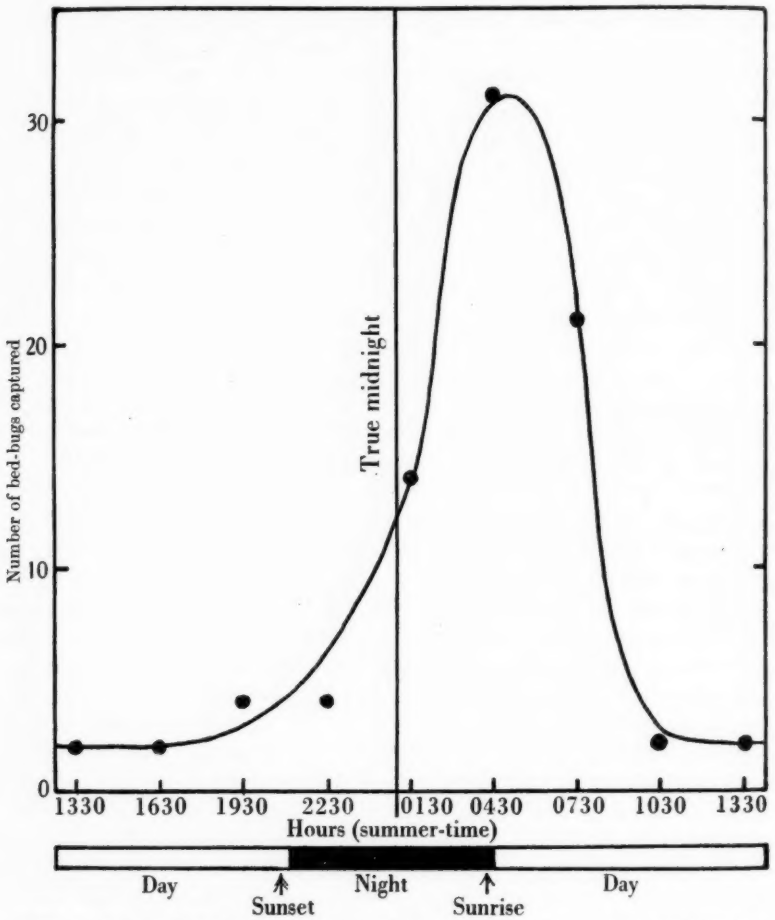

Fig. 3. Activity of bed-bugs in animal house as measured by the number of individuals captured in traps during 3 hr. periods. May 1938.

vented their numbers from increasing sufficiently for experimental purposes.

Though in May the bugs were mostly active at, and after, dawn, the presence of a 60 W. electric lamp left burning all night affected the catch considerably. At this time of year the electric light would give a greater illumination than daylight in the animal room until about 5.30 a.m., after which daylight would be the brighter. As the bug population fluctuated considerably, it was

only possible to compare catches made under natural conditions of illumination and in constant light, provided that the times compared occurred within a very few days of one another. During May, catches were made on seven consecutive nights, during two of which the electric light was left burning. Under natural conditions of illumination the average catch was sixty-five bugs, but on the two nights on which the extra artificial light was shining, only twenty-four bugs were captured in each case. In September a similar comparison was made when the bug population was much smaller. Nevertheless on nine nights without extra light the average catch was 10·7, and on ten nights with the electric light burning the average catch was only 2·3. Approximately the same proportion of fed and unfed bugs was captured on all these nights.

All the bed-bugs do not cease activity completely during the day, though during that period any disturbance such as that caused by men going in and out of the room seems to inhibit activity. Incidentally a similar amount of disturbance at night, as is inevitable when the traps are examined every 3 hr., when not accompanied by extra artificial light does not seem to affect the insect's behaviour, for similar numbers were caught on these nights and on nights when no one entered the room. Several catches were made between 9 a.m. and 6 p.m.; on several weekdays no bugs or only one were caught, but on Sundays when the room was not entered all day as many as nineteen bugs were caught. This was during the part of May 1938 when the average nightly catch was sixty-five.

On one occasion the animal room was kept in complete darkness for a period of 45 hr., from midday on Saturday until Monday morning. The traps were examined throughout the whole period at 3-hourly intervals. The darkness during the day did not in any way upset the rhythm of the bug's activity, for catches made during the day and night were indistinguishable from those made under natural conditions of daylight and darkness.

These various experiments on periodic activity seem to indicate that some inherent rhythm governs the activities of bed-bugs. This rhythm can be upset by altering some of the external conditions such as the light, and even under normal conditions there is some latitude in the bug's behaviour. When it is realized that each individual bug is only active and seeking for food at intervals of 5 or more days, it is interesting to find that the majority are so regular in the periodicity of their activity. It is also clear that laboratory experiments on the behaviour of bed-bugs will have little significance unless the normal periodicity of activity is borne in mind, and unless bugs similar in nutritional state to those found moving spontaneously are used.

POPULATION DENSITY AND LONGEVITY

Extensive experiments have been described by various entomologists in which they have marked individual tsetse flies with recognizable spots of paint, released these flies and recaptured a proportion of them at a later date. Jackson (1937) has made particular use of this method, and has obtained much

valuable information about the movements of wild populations of tsetse flies. I have conducted a small experiment on similar lines with the bed-bug.

One hundred and thirteen bugs captured in the "Demon" traps were marked each with a recognizable pattern of paint spots (see p. 202 above) and, after being weighed, the insects were released in the animal house. Some of the bugs were fed before they were released. Collections were made using the "Demon" traps for 60 days after the last release and Table III shows how many recaptures occurred. In making up this table a bug released on the first of the month and recaptured on the 20th would be equivalent to another, released on the 10th and recaptured on the 30th. It will be seen that bugs were recaptured on twenty-seven occasions. Actually only twenty-one individuals appeared in the traps, but two were caught twice and two others three times each. The last recapture was on the 29th day after release.

Table III. *The recapture of marked bed-bugs*

	Days after release						
	1–5	6–10	11–15	16–20	21–25	26–30	Over 30
Bugs	10	7	4	2	3	1	—

The numbers of bugs used in these experiments are too small to allow of the use of the various formulae devised by Jackson for estimating tsetse fly populations, and they can only give a very approximate idea of the numbers of wild individuals. The frequency with which the bugs were recaptured was insufficient to give further evidence regarding the periodicity of feeding. To arrive at some estimate of the size of the wild population several assumptions must be made, the most important of which concerns the length of life of the marked bugs. None of these were caught more than 29 days after their release. If we assume that on the average they survived half that period, say 15 days, then, as a total of 258 male bugs was caught during the 29 days, if x represents the total number of wild male bugs in the animal room, we arrive at this equation:

$$\frac{x}{258}=\frac{113}{27}\times 2.$$

This makes the adult male part of the wild population to be 2158. This is obviously only a very rough approximation, but it probably gives some idea as to the order of magnitude of the population.

As mentioned above, no bug was captured more than 29 days after its release. In the laboratory many similar bugs lived under similar climatic conditions for months. I think that the shortness of life must be due to the bugs being killed by the rats (see p. 201 above). During the period of the experiment few died from disinfestation of the cages.

If female bugs only survive for 15 days on the average, and if they feed every 5 days, they will each produce about thirty eggs. Incidentally as the rats kill the bugs when they are trying to feed, and as the number of bugs produced

after each feed is about the same whether the female feeds seldom or often, the female suffers the same risk per egg produced no matter what the frequency with which she feeds. More frequent feeding, with consequent more rapid egg production, is an advantage in helping the population to increase rapidly. It should be noted that the period of 15 days is only suggested as a possible *average* length of life, for examinations of their sexual condition suggest that a proportion of the females lived for much longer periods.

These experiments on marking bed-bugs are described rather as a suggestion for an extension of the methods used for the tsetse fly than because they have produced suggestive results.

SUMMARY

A natural infestation of bed-bugs which has existed for several years in an animal room has been studied. The bugs feed on the rats kept in cages in the room.

Methods are described by which the bugs were trapped during their periods of normal activity.

Adult bugs appear to feed on an average once every 5 or 6 days when the temperature is in the region of 20–27° C. (68–80·6° F.). Most of the female bugs lay a continuous series of eggs at the rate of nearly three per day.

Female bugs are not usually fertilized more than once a week in this wild population. In laboratory cultures copulation is much more frequent.

In the animal house the bugs were most active between 3 a.m. and 6 a.m. This activity seems to be controlled by some inherent rhythm.

Some marked bugs were released and the proportion of recaptures to others in the total catch was noted. From this a rough estimate of the population was made. It appears that few adults live more than 29 days as they are probably killed by the rats when trying to feed.

REFERENCES

CRAGG, F. W. (1923). Observations on the bionomics of the bed-bug, *Cimex lectularius* L., with special reference to the relations of the sexes. *Indian J. med. Res.* **11**, 449–73.

HÉRAUD, A. (1916). Destruction de punaises. *Vie agric. rur.* **6**, 296.

JACKSON, C. H. N. (1933). On a method of marking tsetse flies. *J. Anim. Ecol.* **2**, 280–90.

—— (1937). Some new methods in the study of *Glossina morsitans*. *Proc. zool. soc. Lond.* pp. 811–96.

JOHNSON, C. G. (1937). The relative values of man, mouse and domestic fowl as experimental hosts for the bed-bug, *Cimex lectularius* L. *Proc. zool. soc. Lond.* pp. 107–26.

JOHNSON, C. G. & MELLANBY, K. (1939). Bed-bugs and cockroaches. *Proc. roy. ent. Soc. Lond.* (A) **14**, 50.

MELLANBY, K. (1932). Effects of temperature and humidity on the metabolism of the fasting bed-bug (*Cimex lectularius*), Hemiptera. *Parasitology*, **24**, 419–28.

—— (1935). A comparison of the physiology of the two species of bed-bug which attack man. *Parasitology*, **27**, 111–22.

—— (1938). Activity and insect survival. *Nature, Lond.*, **141**, 554.

—— (1939). Fertilization and egg production in the bed-bug, *Cimex lectularius* L. *Parasitology*, **31**, 193.

(*MS. received for publication* 11. I. 1939.—Ed.)

THE MOUTHPARTS AND THEIR FUNCTION IN THE FEMALE MOSQUITO, *ANOPHELES MACULIPENNIS*

By G. G. ROBINSON, M.Sc.

Department of Zoology, Queen Mary College, University of London

(With 9 Figures in the Text)

PAGE

Introduction and historical 212
Material and methods 214
Part 1. The anatomy of the head and mouthparts:
External characters 215
The proboscis 217
Internal skeleton of the head 219
The mouthparts:
The labrum 220
The mandibles 220
The hypopharynx 223
The maxillae 224
The labium 227
Part 2. The functions of the mouthparts:
The labrum 230
The mandibles 231
The hypopharynx 231
The maxillae 232
The labium 233
The piercing mechanism 235
Summary 239
Acknowledgements 241
References 241

Introduction and historical

Dr A. J. Grove of Queen Mary College, University of London, originally made the suggestion that I should give an account only of the piercing mechanism used by the female mosquito during feeding. However, when I searched for a full account of the anatomy of the insect's head on which to base my explanations, I found conflicting descriptions and few useful diagrams. To understand the piercing mechanism it is necessary to have a clear knowledge of the relationships of the mouthparts and of the muscles to the head capsule. An attempt has been made, therefore, to give a description of this anatomy as a preliminary to the discussion of experiments, and,

at the same time, to fill in any gaps in the accounts in the existing literature.

The first serious attempt to describe the anatomy was that of George Dimmock, of Boston, Mass., who in 1881 included an account of *Culex* in his *Anatomy of the Mouthparts and of the Sucking Apparatus of some Diptera.* Although his account is excellent, microtechnique in those days was comparatively undeveloped. The last decade of the nineteenth century saw the momentous works of Manson on filariasis, Ross on malaria and Reed on yellow fever. Prompted by the renewed interest in mosquitoes and aided by modern methods, Nuttall & Shipley (1901–3) in Cambridge and Kulagin (1905) in Moscow independently published accounts of the anatomy of these insects' heads. Kulagin's text which deals with *Culex* as well as *Anopheles* is, on the whole, more detailed, but the diagrams of Nuttall & Shipley are better and more comprehensive. Millet Thompson in Worcester, Mass., followed in 1906 with his *Alimentary Canal of the Mosquito*, but it was not before 1920 that Vogel gave a critical communication on the culicid mouthparts.

The older investigators, one generally finds, express themselves much more fully on the piercing mechanism than their successors of this century, though most of the considerable works are unfortunately somewhat vague. From the earliest times "mosquito bites" must have aroused much interest, but Réaumur in 1734 was one of the first entomologists to describe the process in any detail. Réaumur gives some good illustrations of the appearance of the mosquito during the act of piercing.

With the accumulation of more detailed knowledge concerning insect anatomy, accounts of the piercing mechanism tended to become more involved and not always clearer. For instance, Dimmock (1881) cryptically says that the forward motion of the maxillae is probably due to "the elasticity of the chitin frame of the head".

About the actual wound we have Kulagin's (1905) statement that it is widened by a continuous to-and-fro movement of the stylets during sucking. He further says that, although the maxillae play the leading part in piercing, the "labrum-epipharynx" makes the deepest wound. He does not bring forward evidence for this, but on the face of it the statement is a plausible one.

Vogel (1920) agrees with most of his predecessors when he says that the labella and ligula guide the stylets, but he also says that they adhere to the skin helped by the pseudotracheae which act like the pads on the underside of a gecko's foot. He also gives no evidence for this very theoretical idea.

In 1929 Patton & Evans suggested that the mandibles took no part in piercing, but in the same year the most difficult problem of all was dealt with by Martini, viz. how can such slender stylets penetrate an animal's skin by muscular action at their upper ends? His solution is short and unconvincing, and one of the objects of this paper is to supply an answer to this question.

Material and methods

To provide material for the research, the breeding of insects was undertaken. For the most part the methods used were those worked out by P. G. Shute (1936). A heated room was not available, so a comparatively small thermostatically controlled chamber had to be used, together with suitable breeding cages.

Carnoy's solution was found to be the best fixative. Sectioning was done by the double-embedding (celloidin) method, and the sections were stained in Mallory's triple stain, Ehrlich's haematoxylin and eosin in absolute alcohol, or in iron haematoxylin.

For most of the skeleton the usual potash preparations were found to be sufficient. The muscles were examined in sagittal halves of the head, cut on the microtome. The head was first bleached in chlorine water overnight and afterwards stained in borax carmine. The musculatures of the labium and maxillary palps were elucidated by horizontal sections, and preparations of the whole organ were dissected out and stained in either 1% acid fuchsin or iron haematoxylin. These latter preparations were not mounted, but were examined in cedarwood oil in various positions. Much more was seen this way than in specimens held in a fixed position in Canada balsam.

In order to carry out observations on piercing, unfed female mosquitoes which "looked hungry" were used, i.e. those which, when they were disturbed, raised their palps and started to probe the sides of the cage. Such insects were generally found to bite readily the rabbit provided for them.

For deficiency experiments (removal of one or more mouthparts) mosquitoes which had fed only one or two days previously were selected. They thus had stamina not only to undergo operation under ether, but also successfully to withstand the two or three days' fast subsequent to operation, during which period they were allowed to recuperate.

Many such deficiency experiments were carried out on the insects, and the method used was the same in each case. Each insect in turn was well etherized in a closed tube and put under the binocular microscope for operation. The latter might take as long as 10 min. owing to the difficulty of manipulation, and periodically the insects would show signs of recovery. A small piece of cotton-wool soaked in ether was put beside the insect, which was then covered with an inverted Petri dish. Anaesthesia would then soon become complete for a second time. In this way three or four doses of ether could be given without harming the insect in any noticeable way. Each insect was isolated after operation and a note kept of what had been done to it; when feeding tests had been carried out, or after its death, the insect was always re-examined in alcohol under high power in order to verify the degree of mutilation. The blood sucked by the mosquito is easily seen through its abdominal wall, and recent feeding can instantly be detected in experimental insects.

Other details of experimental methods are best described with the experiments themselves.

PART 1. THE ANATOMY OF THE HEAD AND MOUTHPARTS

External characters

As noted by Nuttall & Shipley (1901), a large part of the surface of the head is covered by the paired compound eyes: the whole of the front half, one might say, is so covered with the exception of areas occupied by the vertex, frons, clypeus, antennal fossae and proboscis. This holoptic condition is unusual for the female dipteran. The curved ventral margins of the eyes approximate closely; the dorsal margins are separated by a narrow area on which is the median coronal suture. This suture runs forward from the occiput and forks at a point just behind the antennae. There is also a median suture, the interantennal suture, between the antennal fossae, which runs back from a line joining the anterior tentorial pits to fork at a point behind the antennae; the two apposed forks thus enclose a small diamond-shaped area whose lateral corners touch the antero-dorsal border of the eye (Fig. 1).

From the front of the head (near the anterior end of the interantennal suture) there is a box-like projection which corresponds to the rostrum of the blow-fly (Fig. 1, *CLP.*). On the anterior ventral part of the latter there is a small invagination which can be named the clypeal fossa: this is figured by Nuttall & Shipley (1901) in their Fig. 8, pl. IX, and Thompson (1906) has mentioned a furrow in a similar position in *Culex*. The floor of this fossa is formed by part of the dorsal wall of the labrum. Just below and at the side of each of the posterior corners of the roof of the rostrum is an elliptical anterior tentorial pit.

With the exception of differences in the degree of projection of the rostrum and in the size and shape of the anterior tentorial pits, the condition of the sutures is similar in Peterson's hypothetical dipteran (1916) and in the females of *Chironomus* and *Mycetophila*. There are two rival interpretations of these structures. Dimmock (1881), Nuttall & Shipley (1901), Kulagin (1905) and Thompson (1906) all call the entire dorsal part of the rostrum clypeus (its ventral wall is part of the gular region of the head); Theobald (1901–3), Christophers (1933) and Barraud (1934) figure, in addition, the area traversed by the interantennal suture as frons. According to the interpretations of these authorities the median interantennal suture cannot be fundamentally connected with the coronal, and must be a secondary acquisition of the frons—an almost unique occurrence. Actually it will be seen from Fig. 1 that the interantennal and coronal sutures are separated by the small diamond-shaped area. The other interpretation is that of Peterson (1916) who calls the interantennal suture epicranial (i.e. a continuation of the coronal) in his hypothetical dipteran whose condition is like that of the female mosquito. On this hypothesis the interantennal area becomes vertex and the dorsal wall of the rostrum becomes fronto-clypeus. The articulation of the latter with the head would now be marked by the facial suture; according to the alternative interpretation this suture would be epistomal.

To sum up, we have two interpretations of the identity of the interantennal area and of the dorsal wall of the rostrum. Peterson names the areas vertex

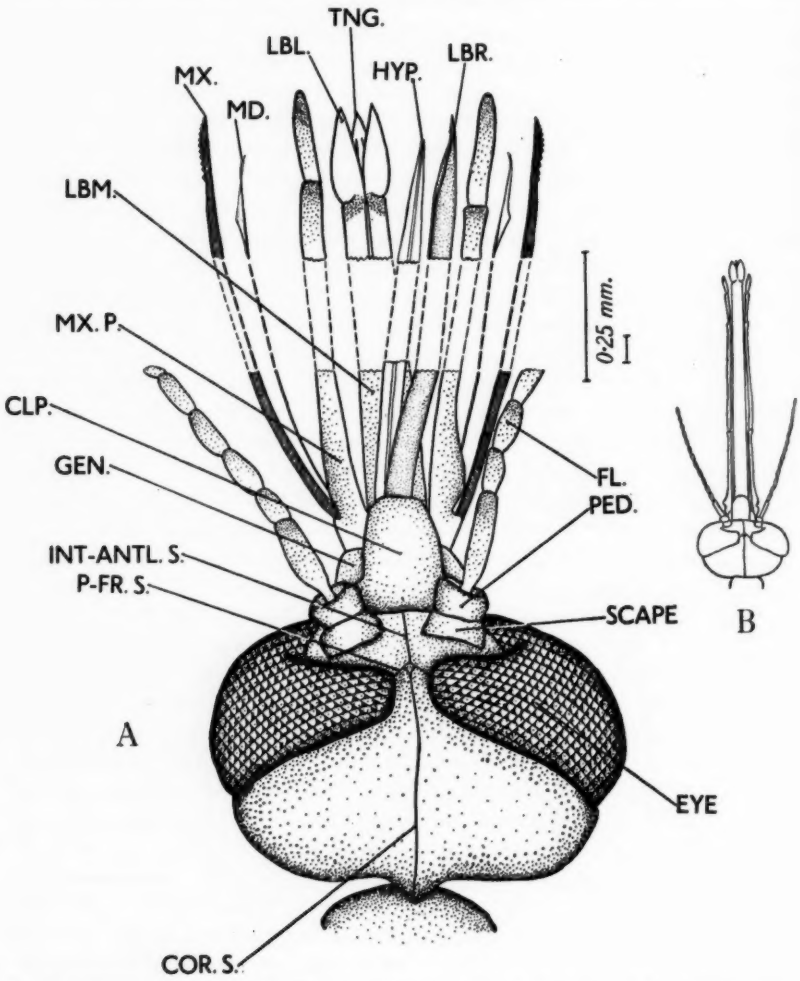

Fig. 1. A dorsal view of the head with the mouthparts displayed. A, shortened for spatial reasons; B, natural proportions. *CLP.* clypeus; *COR.S.* coronal suture; *FL.* flagellum; *GEN.* gena; *HYP.* hypopharynx; *INT-ANTL.S.* interantennal suture; *LBL.* labellum; *LBM.* theca of labium; *LBR.* labrum; *MD.* mandible; *MX.* galea of maxilla; *MX.P.* maxillary palp; *PED.* pedicel; *P-FR.S.* postfrontal suture; *TNG.* ligula.

and fronto-clypeus respectively, and most other authors name the areas frons and clypeus respectively. However, for what it is worth a compromise is possible, and in this present paper the interantennal area will be included as

part of the vertex (agreeing with Peterson), and the dorsal wall of the rostrum will be called clypeus (agreeing with most other authors, including Snodgrass (1935), who actually gives the Diptera the special characteristic of a clypeo-labral muscle as distinct from the fronto-labral muscle of other insects). What then has become of the frons? Imms (1934) remarks that the dipteran frons may be of limited extent, and the condition in the mosquito suggests that the two prongs of the facial fork have come closer and closer together until they have met to form the median interantennal suture. The merging of the prongs of the facial fork can be thought of as the result of the invasion by the interantennal vertex (originally quite a small area bounding the upper part of the frons) of the area formerly covered by the frons. The small diamond-shaped area mentioned above shows the position once occupied by the median ocellus (Thompson (1906), for *Culex*, figures the ocellar nerve). The small fork from the coronal suture (Fig. 1, *P-FR.S.*) mentioned above will be called the post-frontal suture—similar to that seen in Dermaptera and Plecoptera. According to Peterson's figure *Mycetobia* also has such a suture, and it is suggested that he might have added this to the characters of his hypothetical dipteran.

The remaining "sclerites" of the head are undefined by sutures which are present in the more generalized insects. There is a preocular bulge of the head proper which Kulagin (1905) calls the "cheek"; Thompson (1906) in his pl. 13, fig. 4, labels it as "gena", and Christophers (1933) calls it the "anterior part of the postgena". Thompson's terminology will be followed, but it is difficult to give the area its true value, as, in the mosquito, displacement has taken place owing to the prognathous condition and to the large development of the compound eyes.

Lying laterally and below the occipital foramen on each side is a posterior tentorial pit which is relatively very large and shaped like a trumpet.

The gular region, the anterior part of which forms the ventral wall of the rostrum, is membranous as also is the interantennal vertex.

The proboscis

The mouthparts consist of the labrum, a pair of mandibles, the hypopharynx, a pair of maxillae and the labium. The latter is a proboscis-like structure about four times as long as the head from the lower, anterior region of which it projects forwards (Fig. 1B). On its upper or dorsal side the labium has a deep groove and this houses all the other mouthparts which are arranged in a bundle as follows: the mandibles cling to the ventro-lateral edges of the labrum: underlying these medianly is the hypopharynx: the stems of the maxillae lie close to the lateral walls of the hypopharyngeal midrib and their blades curve outwards and upwards near to the mandibles. All these mouthparts lie in the labial gutter whose edges in the distal half of the proboscis meet over the dorsal side of the labrum. Vogel (1920) gives a good diagram of a transverse section of the proboscis (Fig. 2). For purposes of clear representation in this diagram Vogel has shown the stylets aggregated in a very loose

bundle, i.e. not touching one another. He omits to say that, at least in the distal half of the resting proboscis, they are in close apposition to one another.

Proximally the elements of the proboscis unite with the rostrum opposite the middle of the clypeus. Here the first part of the stomodaeal tube (buccal cavity) is formed by fusion of the edges of the inner wall of the labrum and the hypopharynx. The outer wall of the labrum joins the clypeus. The ventral wall of the labium passes over into the gular region of the head, and the dorsal wall

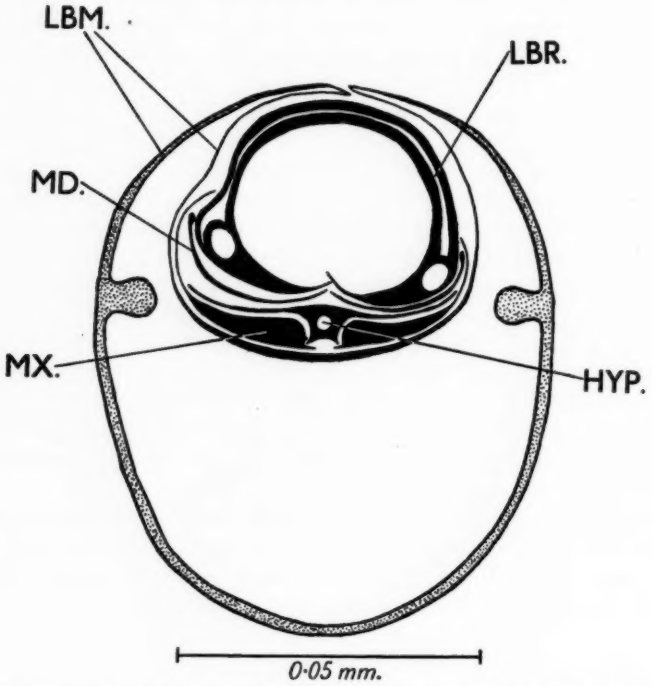

Fig. 2. A transverse section of the middle region of the proboscis (after Vogel, 1920). *HYP.* hypopharynx; *LBM.* theca of labium; *LBR.* labrum; *MD.* mandible; *MX.* galea of maxilla.

(the gutter-like groove) is limited by the base of the hypopharynx. The mandibles above and the maxillae below pass into the head close to one another and at the side of the clypeus between it and the gena.

When at rest the distal extremities of the piercing stylets (labrum, mandibles, hypopharynx and maxillae) lie hidden by the terminal lobes of the labium. Prior to piercing they are protruded.

J. B. Smith (1890) wishes to prove that labrum, hypopharynx and labium of the Diptera do not take part at all in the composition of the proboscis, which according to his view is made up of modifications of the maxillae. Little notice seems to have been taken of his paper.

Internal skeleton of the head

The tentorium. Each of the anterior tentorial pits sends backward an open tube (the anterior arm) about 40 microns in diameter (Fig. 3, *A.T.*). This bears posteriorly a dorsal spine which represents the atrophied dorsal arm (Fig. 3, *D.T.*). Each of the posterior trumpet-like pits sends forward a posterior arm (about 20μ in diameter) to join the anterior arm in a somewhat artificial-looking joint (Fig. 3, *P.T.*). The posterior arm bears an extremely slender dorsal process which comes off anteriorly to pass back to the occiput with

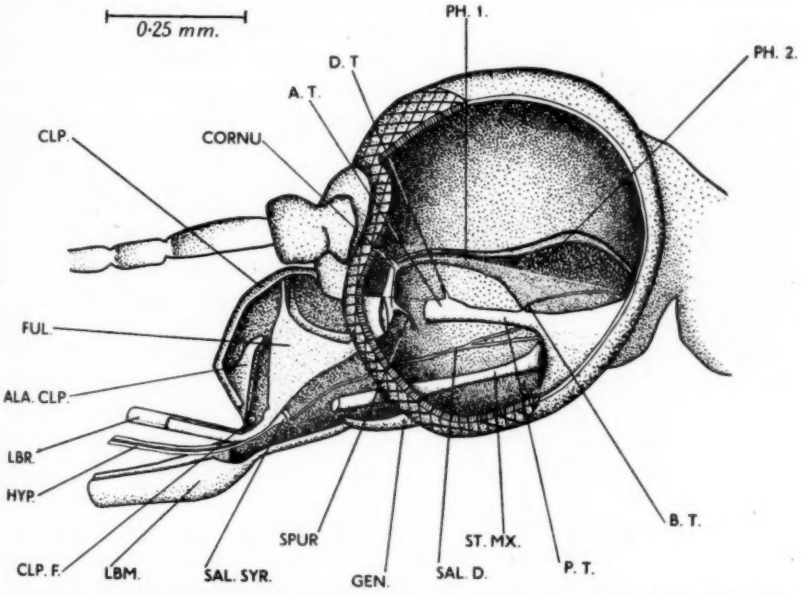

Fig. 3. The head displayed from the left side to show the left tentorium and stipes. (For clarity a part of the anterior tentorium has been removed.) *ALA.CLP.* ala of clypeus; *A.T.* anterior tentorial arm; *B.T.* body of tentorium; *CLP.* clypeus; *CLP.F.* clypeal fossa; *CORNU.* fulcral cornu; *D.T.* dorsal tentorial arm; *FUL.* fulcral plate; *GEN.* gena; *HYP.* hypopharynx; *LBM.* theca of labium; *LBR.* labrum; *PH.* 1. anterior pharynx; *PH.* 2. posterior pharynx; *P.T.* posterior tentorial arm; *SAL.D.* salivary duct; *SAL.SYR.* salivary syringe; *ST.MX.* stipes of maxilla.

which it fuses (Fig. 3, *B.T.*). These processes represent the body of the tentorium. The ventro-mesial border of the anterior arm of the tentorium has a solid cuticular ridge which is continued forward in front of the anterior tentorial pit as a lateral shelf inside the gena (Fig. 5, *S.*). It corresponds to the "fronto-clypeal apodeme" in *Culicoides* (Jobling, 1928) and in some individuals of *Anopheles* this shelf is poorly developed. Kulagin (1905) figures it in cross-section.

Clypeal apodemes. On the inner side of the anterior face of the clypeus

there is a pair of structures each of which has the shape of a blunt thorn (Fig. 3, *ALA.CLP.*). Nuttall & Shipley (1901) do not mention these structures; Kulagin (1905) gives a good description and figure and calls them "laminae frontales"; Thompson (1906) figures them and refers to them as "alae" of the clypeus. Kulagin mentions their occurrence in the ant.

The mouthparts

The labrum. The labrum is the most dorsal of the piercing stylets and for

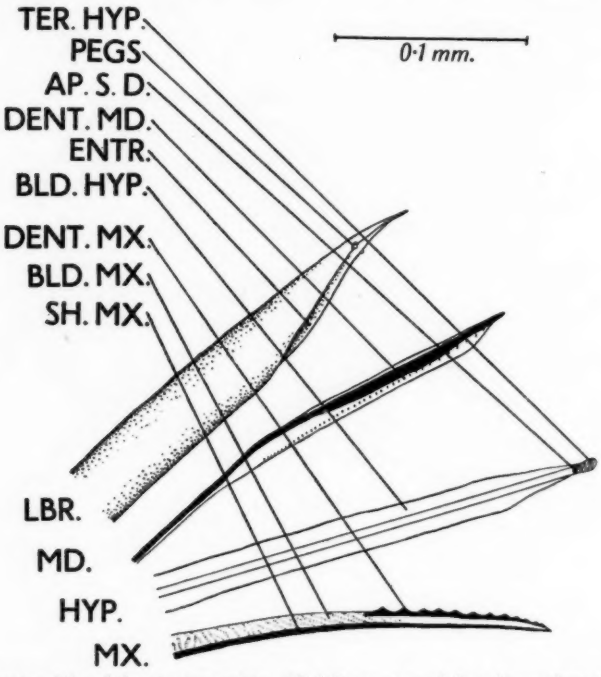

Fig. 4. The extremities of the piercing stylets. *AP.S.D.* aperture of the salivary duct; *BLD.HYP.* wing of hypopharynx; *BLD.MX.* blade of galea with striations; *DENT.MD.* mandibular denticles; *DENT.MX.* maxillary denticles; *ENTR.* entrance to food channel; *HYP.* hypopharynx; *LBR.* labrum; *MD.* mandible; *MX.* galea of maxilla; *SH.MX.* shaft of galea. *TER.HYP.* termination of hypopharynx.

the greater part of its length it is enclosed by the labial groove, but proximally it projects a little from this cavity. Like the labium it can be considered as a hollow cylinder, the ventral wall of which is traversed by a straight longitudinal groove, the food channel (Fig. 2, *LBR.*); but here the groove is so deep that not much of the cavity of the original cylinder is left and the sides of the groove are touching except for a short distance at the tip of the organ and at its base, where the hypopharynx forms a ventral closure. At the tip the stylet is sharpened off ventrally like a quill pen and consequently the groove is open

(Fig. 4). Just below the point there is a pair of small pegs which may function as sense organs.

At its base the outer wall of the labrum fuses with the dorsal wall of the clypeus. As stated elsewhere, the floor of the clypeal fossa is formed by the most proximal part of the outer wall of the labrum. The inner wall here passes over insensibly to the buccal cavity. Thus the labrum is firmly connected with the head of the insect.

There have been two controversies about the labrum of the mosquito. Vogel (1920), it appears, was the first to point out that the labral canal, or food channel, was closed ventrally by the apposition of the edges of the labral groove. (In *Culex* there is a locking mechanism.) Until his time, other authors figured the labrum as having an open ventral groove closed only by close appression of the hypopharynx. Secondly, the term "labrum-epipharynx" is almost universally used for the labrum. Becher (1882), Kräpelin (1882), Thompson (1906) and Vogel (1920) interpret this dorsal stylet as wholly labral. The majority of other authors refer to the food channel as bounded by the epipharynx, and Kulagin (1905) even says that the "epipharynx" is not joined to the "labrum" along its length and that it should be considered as an independent stylet. Actually there is no evidence that the epipharyngeal part of the gut (buccal cavity) has extended into an area primitively occupied by the inner wall of the labrum, and the logical view is that the dorsal stylet is wholly labral. Most text-books in English continue to depict the "labrum-epipharynx" as a "ventrally open gutter closed along its length by the hypopharynx". (Not Snodgrass, however.)

In a transverse section of the labrum the internal (haemocoelar) lumen can be seen to be occupied by a pair of protoplasmic strands lying laterally (Fig. 2). Vogel (1920) interprets these as nerves which terminate distally in sense organs in the position of the small pegs. It is interesting to note that Macgregor (1931) records the ability of *Aedes* and *Culex* to select a desired liquid by means of a sense located in the tip of the labrum.

The labrum has paired elevator muscles taking origin on the ventral curve of the alae of the clypeus and inserted on the sides of the inner wall of the labrum at the level of the clypeal fossa (Fig. 6, *LEV.LABR.*). Dimmock (1881) mentions the labral muscles going from the base of the labrum to the roof of the clypeus; Nuttall & Shipley (1901) do not describe them and Kulagin (1905) and Thompson (1906) call them "muscles of the epipharynx". A median retractor of many separate bundles originates on the dorsal wall of the clypeus and is inserted on the roof of the clypeal fossa (Figs. 6, 7, *RET.LBR.*). Nuttall & Shipley (1901) briefly mention this while Kulagin (1905) describes the "fan-shaped levator labri" as inserted on "the base of the lip" and originating on "the front and partly also the back part of the forehead". Thompson (1906) more accurately describes the muscles as "inserted on the boss of chitin at the base of the labrum". Snodgrass (1935) considers this muscle as a special character of the Diptera as opposed to the fronto-labral muscles of other insects.

The mandibles. The mandibles are the most slender of the mouthparts. Each consists of an elongate, slightly trough-shaped strip of chitin, the distal end of which is flattened and expanded into a scimitar-like blade. The distal half of the outer edge of the latter bears a row of extremely fine teeth, the number of which varies from thirty to fifty; the apices of the teeth are about $2\frac{1}{2}\mu$ apart (Fig. 4). Nuttall & Shipley (1901) mention the number as thirty-one and MacCloskie (1888) quotes M. S. Morgan of Princeton as saying that there are forty-two teeth on the mandible. These statements were apparently made after examination of the mandibles of only one individual.

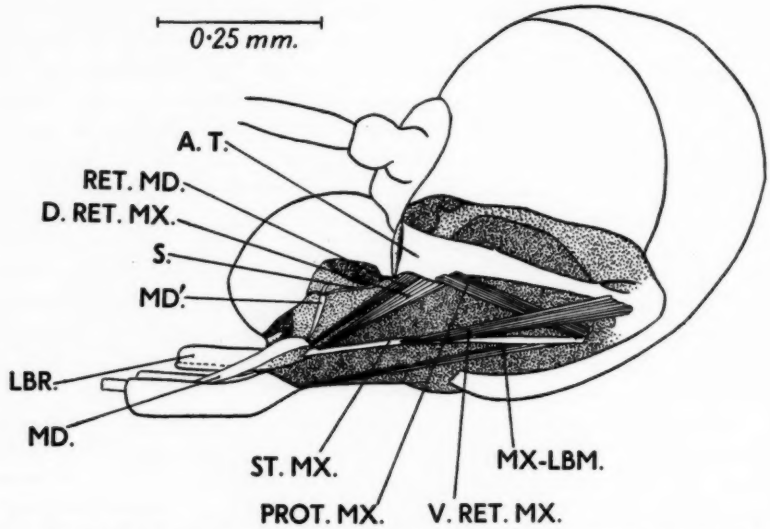

Fig. 5. The head from the left side, showing the mandibular and maxillary muscles. *A.T.* anterior arm of tentorium; *D.RET.MX.* dorsal retractor of maxilla; *LBR.* labrum; *MD.* mandible; *MD'.* mandibular suspensorium; *MX-LBM.* maxillo-labial muscle; *PROT.MX.* protractor of maxilla; *RET.MD.* retractor of mandible; *S.* genal shelf; *ST.MX.* stipes; *V.RET.MX.* ventral retractor of maxilla.

Proximally the mandibles originate at the side of the rostrum between the latter and the base of the maxillary palp. Each is supported at its base by articulation with the mandibular suspensorium (Fig. 5, *MD'.*). This consists of a slender slip of chitin shaped somewhat like a wooden tent-peg, the pointed end of which joins the proximal end of the mandible and the head of which fits into a recess in the internal genal shelf (Fig. 5, *S.*). This suspensorium is mentioned only by Kulagin (1905) who names it the "transverse rod". Jobling (1928) describes a similar though much better developed structure and arrangement in *Culicoides* (the "mandibular condyle" and "fronto-clypeal apodeme"). As already noted, the mandibles lie between the ventro-lateral edges of the labrum and the wings of the hypopharynx.

The mandibles have only retractor muscles running from the base of the mandible to originate on the ventral surface of the anterior tentorial arm (Fig. 5, *RET.MD.*). Kulagin (1905) and Thompson (1906) are the only authors who mention the mandibular muscles.

The hypopharynx. The hypopharynx is a long, thin, lamelliform extension from the ventral wall of the buccal cavity. On its underside is a median rib, quadrangular in cross-section, containing a similarly extended portion of the salivary duct (Fig. 2, *HYP.*). At the distal extremity both lamella and midrib grow narrow and the latter forms the sharp point of the organ, the orifice being subterminal (MacCloskie, 1888) as seen in Fig. 4. It is thus in a protected position for penetration. Proximally the hypopharynx clings closely to the

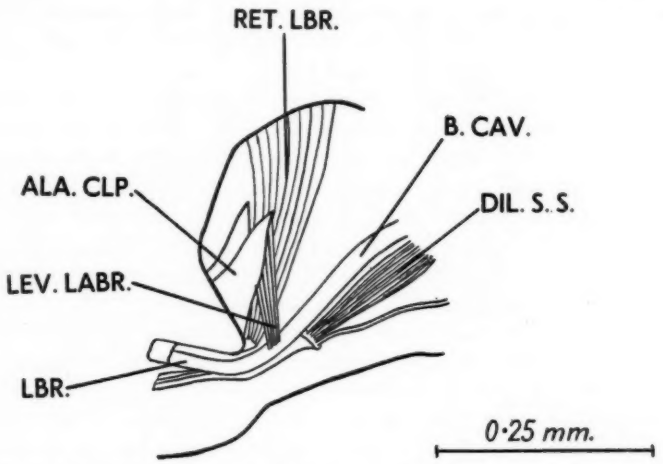

Fig. 6. The muscles of the labrum from the left side. *ALA.CLP.* ala clypealis; *B.CAV.* buccal cavity; *DIL.S.S.* dilator of salivary syringe; *LBR.* labrum; *LEV.LABR.* elevator of labrum; *RET.LBR.* retractor of labrum.

labrum; the labral tube here is not complete ventrally, and the hypopharynx forms a ventral closure and passes over into the floor of the buccal cavity. As already noted, the hypopharynx lies medianly between the maxillae and the labrum (Fig. 2).

At the base of the hypopharynx and close to the ventral surface of the buccal cavity is the salivary syringe which is a local development of the common salivary duct into a bell-shaped structure (Fig. 3, *SAL.SYR.*). The apex of the bell points anteriorly and is continuous with the midrib. The rim of the bell points posteriorly and its mouth is closed by an elastic membrane to the centre of which the common, undilated salivary duct connects. The point of connexion is where the dilator muscles of the salivary syringe are inserted (Fig. 6, *DIL.S.S.*). These are a pair of stout, fusiform muscles which

run ventrally to the buccal cavity to take origin on the spurs of the fulcrum. Most authorities mention them as "hypopharyngeal muscles", and Kulagin (1905) points out the very obvious mistake made by Léon (1904) who shows the muscles originating on the ventral wall of the head-capsule. During biting the salivary syringe pumps saliva along the duct in the hypopharynx and into the victim's wound.

From the paired salivary glands in the thorax two ducts lead forward which continue into the head to join the common salivary duct beneath the anterior pharynx (Fig. 3, *SAL.D.*).

The description of the labrum deals with the common and erroneous idea that the hypopharynx forms the ventral wall of the food channel for the whole of its length. The hypopharynx has no muscles apart from those which work the salivary syringe; thus it has very little freedom of movement as it is firmly united to the head like the labrum, and can, therefore, take only a passive part as the fascicle penetrates the host's skin.

The maxillae. The maxillae are somewhat reduced. Thus the cardo and lacinia are almost non-existent, being represented by small membranous areas not always discernible. The stipes, in common with those of other Diptera, is embedded in the head below the alimentary canal and preserves its primitive association with the posterior tentorial pit. It consists of a solid rod of chitin stretching from the occiput to the base of the clypeus. Its occipital end is boot-shaped (Figs. 3, 7, *ST.MX.*). Distally it articulates with the long, lancet-like galea and gives rise directly to a five-segmented palp (Fig. 7); the palpifer is absent in most Diptera, but in *Anopheles* there is indication of a remnant of it (see below). The palps are slightly longer than the proboscis. The first segment of each is small and fused to the second; the next smallest is the terminal segment, then come segments 4 and 2, the third being the longest (Fig. 1B). Christophers (1933) mentions that the ratio of the lengths of palpal segments 5 and 4 constitutes the "palpal index" (0·3–0·7).

The galea, as noted above, is lancet-like. It consists of a rod of chitin with a trough-shaped lamella adhering to its outer edge, the cross-section resembling that of a straight razor (Fig. 2, *MX.*). The lamella has fine, transverse, occasionally anastomosing striations (Fig. 4, *MX.*). At the extreme end of the galea the lamella narrows and bears about a dozen lateral teeth which are small at the tip of the galea, increase in size rapidly farther back, the proximal two or so decreasing again very slightly. The teeth near the extremity are backwardly directed (Fig. 4). Kulagin (1905) wrongly states that the lamella disappears before the end of the galea and that it is the chitinous rod which bears the "papillae" directly. Nuttall & Shipley (1901) also state that it is the rod which bears the teeth. Dimmock (1881) for *Culex* rightly describes the "papillae" as being on the lamella. The galea unites with the ventro-mesial part of the palp-base and the stipes articulates at this junction (Fig. 7).

In a paper by Roubaud (1928) there is much interesting information on the variation of the maxillary index (average number of teeth on a single maxilla

in the individual) in *Anopheles maculipennis* in various geographical regions of the world. Following statistical evidence, the author tries to relate differences of maxillary index of different faunas of this species to differences in the thickness of the skin of their respective hosts, but this theory has not met with general acceptance.

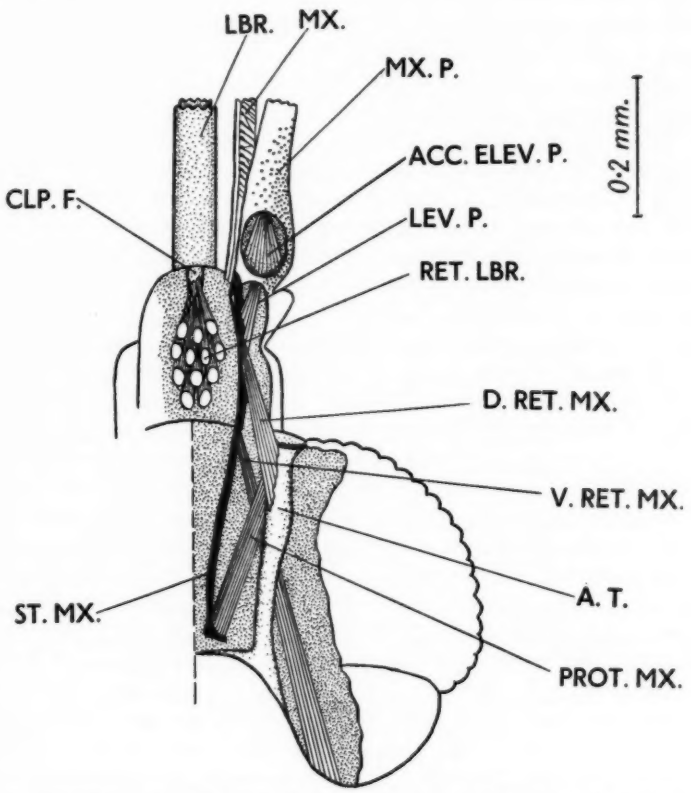

Fig. 7. The muscles of the right maxilla from above. The labral retractor is also shown. *ACC. ELEV.P.* accessory elevator of palp; *A.T.* anterior arm of tentorium; *CLP. F.* clypeal fossa; *D.RET.MX.* dorsal retractor of maxilla; *LBR.* labrum; *LEV.P.*, elevator of palp; *MX.* galea; *MX.P.* maxillary palp; *PROT.MX.* protractor of maxilla; *RET.LBR.* retractor of labrum; *ST.MX.* stipes; *V.RET.MX.* ventral retractor of maxilla.

As already noted, the maxillae lie along the floor of the labial gutter separated from each other by the midrib of the hypopharynx (Fig. 2).

Although Dimmock (1881) says that "the maxillae have no protractor muscle", Nuttall & Shipley (1901) mention "certain fibres which...would obviously serve to protract the maxillae". Actually each maxilla is protracted by a muscle which takes origin on the ventral surface of the anterior arm of

the tentorium and which is inserted on the outer side of the base of the stipes (Figs. 5, 7, *PROT.MX.*). This corresponds to the protractor of the "lower jaws and labium" of Kulagin (1905) whose figures are poor, incomplete and inaccurate. Thompson (1906) describes and figures these muscles well for *Culex.*

Thompson (1906) likewise describes and figures well the two retractor muscles of the maxilla and calls the dorsal retractor the "double retractor" because it sends fibres to the dorsal angles of the base of the labium. Kulagin (1905) gives a complicated account from which may be gleaned the fact that he calls the dorsal and ventral retractors "flexor" and "extensor" respectively. The dorsal retractor (Figs. 5, 7, *D.RET.MX.*) originates on the lower mesial border of the anterior arm of the tentorium, and is inserted on the anterior, backwardly pointing spur of the stipes; the ventral retractor (Figs. 5, 7, *V.RET.MX.*) originates on the occiput near the outer margin of the posterior tentorial pit and is inserted on the middle stretch of the stipes. Dimmock (1881) mentions the ventral muscle only, Nuttall & Shipley (1901) figure and briefly mention the muscles.

Kulagin (1905) says that the maxillary palps have two main muscles: an extensor which originates on the "lower rodlet of the chitin-frame" and is inserted on the "outer edge of the palp"; and a flexor which originates "on the edge of the head where the tube of the chitin-frame shows an opening" and is inserted on the "inner edge of the palp". In addition "in each individual palp segment are two muscles except for the last segment in *Culex pipiens*". Now in general the muscles of the insect palp are intrinsic muscles of the maxilla and normally originate within the maxilla—actually on the stipes. It seems unlikely then that a palpal muscle would be inserted on the tentorium, as Kulagin alleges. In the present investigation no muscle corresponding to Kulagin's "flexor palpi" has been found, but the dorsal retractor muscle of the maxilla is inserted on the stipes where that member is joined by the galea and palp (Figs. 5, 7, *D. RET.MX.*). A muscle which may correspond to Kulagin's "extensor" originates on the fore end of the stipes and is inserted dorsally in the base of the palp: this would act as an elevator (Fig. 7, *LEV.P.*). The insertion point of this muscle occurs just distally to a constriction which marks off the main body of the palp from a small basal bulge (Fig. 7). Normally the elevator is inserted on the basal palp-segment, and, therefore, it seems that the small bulge represents a remnant of the palpifer. It bears a striking resemblance to the first segment of the palp in *Culicoides pulicaris* shown in Jobling's account (1928), but differences in musculature preclude the possibility of homology.

One other muscle only was found in the palp, the accessory elevator, and on this interpretation is the one originating ventrally in segment one and inserting dorsally in segment two (Fig. 7, *ACC.ELEV.P.*). As stated above, these two segments are fused one with the other, so the muscle is small and not of much use.

The labium. The posterior part of the labium shares the ventral surface of the head with the gular element, but the extent of the areas is not defined by sutures. However, a membranous crevice, the labial suture, separates this posterior part from the theca which has the form of an elongate, slightly tapering cylinder, about four times as long as the head (Fig. 1B). Its dorsal (oral) aspect has a groove, the edges of which touch each other in the distal half of the organ and whose depth is about half the diameter of the cylinder (Fig. 2). Dimmock (1881) describes the labium (theca) of *Culex* as having a delicately annulated structure, and Vogel (1920) for the same genus (spp. *pipiens* and *annulatus*) says, "the surface of the middle section of the labium ...shows a system of fine, reticular, dark, aligned chitin ridges between which soft, pale chitin is found". In *Anopheles maculipennis* on the contrary such striations are found only in the proximal and distal regions of the theca and not at all in the middle region. These striations, poorly developed in comparison with those of *Culex*, seem to be not ridges but crevices in the labial integument arranged at right angles to the long axis of the organ. Vogel thinks that this structure is of importance in the extension of the kinked theca after feeding; Patton & Cragg (1913) make the more likely suggestion that it may very well facilitate the sharp bending of the chitinous walls of the organ during the act of penetration.

By enclosing the remaining mouthparts, except the maxillary palps, in its groove, the theca forms the mosquito's proboscis (Fig. 2).

At its extremity the theca bears a little pointed tongue, the ligula (Figs. 1, 8, 9A, *TNG.*), and on each side of this a movable labellum (Figs. 1, 8, 9A, *LBL.*). The ligula is a direct continuation of the thecal groove but its distal half is flattened. Ventrally it has two longitudinal flaps (Fig. 8) and its tip has a group of fine hairs (Fig. 9A). The labella are bead-shaped lobes a little longer than the tongue and somewhat hollowed on their mesial aspect so that they clasp the extremities of the piercing stylets (Fig. 8). Ventrally each labellum has at its base a small **S**-shaped skeleton near the median line (Fig. 9A, *LBL.SKL.*). The mesial face of each labellum has its dorsal and ventral margins thrown into a number of longitudinal lamella-like folds which curve in and press on the ligula and mouthparts (Figs. 8, 9A, *PS-TR.*). They have been compared by Vogel (1920) to the pseudotracheae of the muscids. At the tip the inner face of each labellum has a dense brush of fine hairs (Fig. 9A).

Kellogg (1899) and many others homologize the mosquito's labella and ligula with the paraglossae and fused glossae respectively of the more primitive insects, but Crampton in a paper in 1925 presents good reasons for supposing that the labella are the reduced labial palps and that the theca represents the fused palpigers. We should have to be prepared to reconcile this view with the fact that the labellar muscles take origin in the theca and that in *Culex* the latter has also a special set of muscles of its own. Snodgrass (1935) criticizes Crampton's homologizing of the labella with the labial palps because "the lobes of the fly-labium have usually each only one muscle inserted upon it",

whereas palpi usually have antagonistic muscles. Vogel (1920) for *Culex* and this paper for *Anopheles maculipennis* show that the labial lobes of these insects have antagonistic muscles like typical palpi.

The muscles of the theca are restricted to the distal half of this organ, and are related exclusively to movements of the labella. The largest muscles are the extensors of the labella; each of these is inserted on the outer side of the base of the labellum and proximally is divided into about eleven strands originating on the lateral walls of the theca (Fig. 9B, *EXT.LBL.*). The flexors are much smaller and much shorter muscles. Each consists of from four to ten

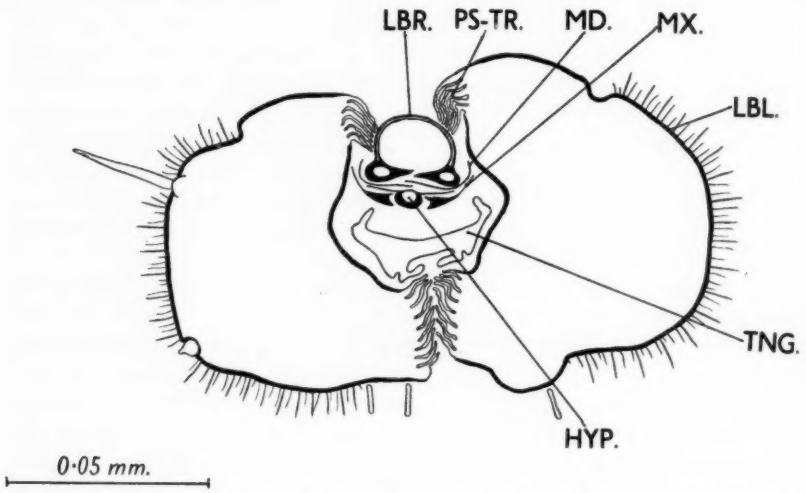

Fig. 8. A transverse section through the labellar region of the proboscis. (Stylets separated owing to fixation.) *HYP.* hypopharynx; *LBL.* labellum; *LBR.* labrum; *MD.* mandible; *MX.* maxillary galea; *PS-TR.* pseudotracheae; *TNG.* ligula.

strands which originate on the dorsal wall of the theca and are inserted by means of a long tendon on the inner border of the labellum running close to the outer arm of the "S" of the basal labellar skeleton (Fig. 9A, *TEN.FLX.*). Nuttall & Shipley (1901) mention a few muscle strands in the labium. Vogel (1920) mentions for *Culex* certain V-shaped muscle fibres entirely within the theca, but these are not present in *Anopheles maculipennis*.

Kulagin (1905) mentions a pair of muscles running from the "lower chitin-skeleton" to the base of the "lower lip"; Thompson (1906) descriptively calls this muscle the "maxillo-labial" muscle, and it originates on the posterior end of the stipes and is inserted on the extreme base of the theca (Fig. 5, *MX-LBM.*). Here we see specialization in the origin of a labial muscle on the maxilla instead of on the cranium.

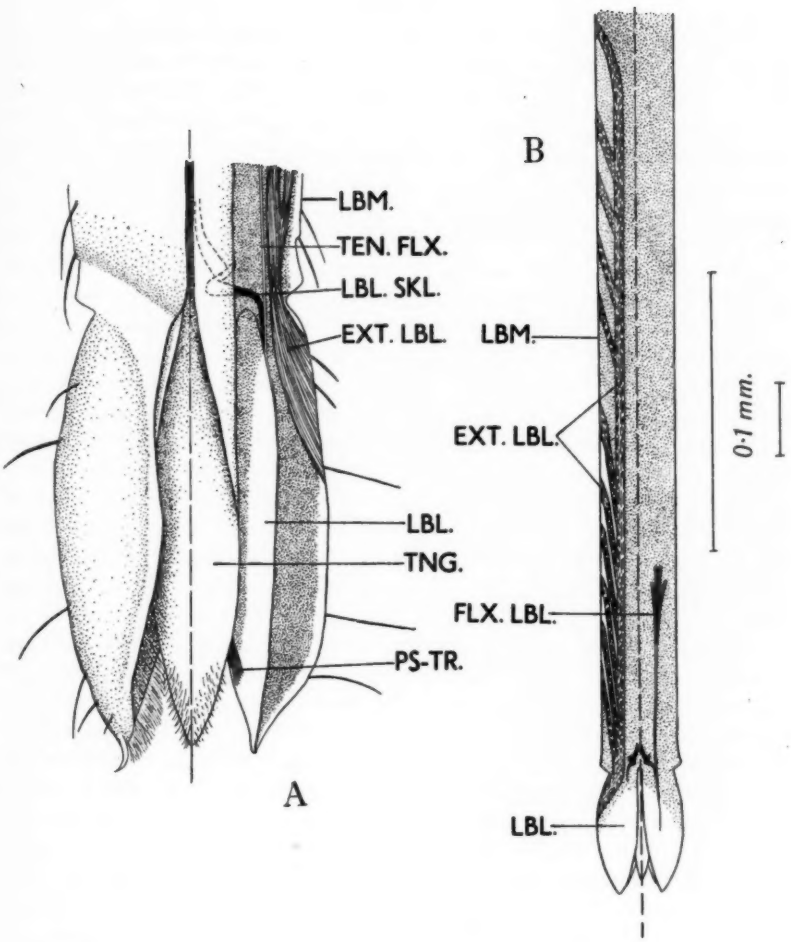

Fig. 9. The muscles of the labium. A. Dorsal view of the labella and ligula. (The left labellum cut away horizontally to show muscle-attachments.) B. The distal half of the theca, dorsally, showing the right extensor muscles and the left flexor muscles. *EXT.LBL.* extensor of labellum; *FLX.LBL.* flexor of labellum; *LBL.* labellum; *LBL.SKL.* basal labellar skeleton; *LBM.* theca; *PS-TR.* pseudotracheae; *TEN.FLX.* tendon of flexor of labellum; *TNG.* ligula.

Part 2. The functions of the mouthparts

The labrum

The function of the labrum, of course, is to act as the food channel through which blood is transferred from the tissues of the host to the alimentary canal of the insect. However, as was indicated above, very firmly rooted is the notion that the hypopharynx plays an essential part in the formation of that food channel. Not only is it ascertainable anatomically that this is not the case, as Vogel pointed out as late as 1920, but also it can be proved experimentally that the hypopharynx is not essential to the actual taking in of food. Thus a female mosquito from which the hypopharynx had been nearly wholly removed was found to be able to draw up an exposed liquid normally. Unfortunately time did not allow of a similar insect being observed feeding in the normal manner on the mammalian host, but it seems inconceivable that it should not possess the ability to do so. However, it is hoped that future experiments will be undertaken. Supposing that the hypopharynx was as a rule a necessary part of the food channel, it seems unlikely in this experimental case, when the stylet was not present, that the mandibles and maxillae could have successfully taken its place. It was mentioned above that the labral groove is open for a very short distance at its base and that here the food channel is bounded ventrally by the hypopharynx. It is probable that too hasty examination of this part only of the proboscis, coupled with imperfect technique, was responsible for the original error in the description of the food channel.

Dimmock (1881) remarks that "at its base the labrum sends a chitinous support beneath the clypeus where it separates more from the epipharynx and has its own muscles, indicating that the labrum has a degree of motion independent of the epipharynx". In other words, the outer wall of the labrum has "a degree of motion independent" of the inner wall. This assumption on Dimmock's part has been found to be correct in the present investigation, and the independent motion comes about apparently by means of alternate contraction of the retractor and levator muscles of the labrum (Fig. 6). When in a partially anaesthetized insect the labrum is separated from the rest of the mouthparts it may often be seen, viewed from the side, to assume periodically the characteristic shape of a slight sigmoid curve and then (on relaxation of the muscles) to resume its usual straight appearance. It seems that this kind of movement would be useful in directing the labrum to the desired blood vessel during the act of piercing, and presumably for efficiency in this guidance the terminal, peg-like sense organs on the labrum would be indispensable, none of the other stylets being innervated in any way.

The tip of the labral tube, sharp like a hypodermic needle, facilitates its entry into the host's skin, and in addition to the obvious importance of the labrum as the food channel, it will be argued below that there is a special significance in its tubular structure relating to the mechanism of piercing.

The mandibles

Patton & Evans (1929) suggest that the mandibles are unimportant in the piercing mechanism; in view of their lack of protractor muscles and the slender build of these appendages this seems highly likely and indeed is generally accepted. However, deficiency experiments ought to be carried out as a verification. Macgregor (1931) says that both mandibles and maxillae can be used by the mosquito to "dislodge particles from the tip of the proboscis". When the insect is at rest the wide terminal blades of the mandibles act as a cap to the entrance of the labral canal and serve for its protection during penetration. During sucking this cap is apparently withdrawn by means of the retractor muscles.

The hypopharynx

There can be little doubt that it is the secretion of the salivary glands of the mosquito which causes irritation in the host's tissues subsequently to a bite. This, however, is incidental, and in the long run is rather a hindrance than a help to the insect in whose interest it lies to causes as little annoyance as possible to the animals which supply its meals.

Fülleborn (1908) mentions that "when one pushes the proboscis sheath aloft along the stylet bundle, one brings simultaneously a drop of fluid on the point of the bundle". In nearly all cases when mosquitoes were anaesthetized with ether, a similar "drop of fluid" was seen to emerge from between the labella. It is easily seen (and well known clinically) that at one stage in progressive anaesthesia convulsive contractions of the muscles take place; the muscles of the salivary syringe being no exception to this rule, it is evidently their "involuntary" contractions which force this drop from the tip of the hypopharynx.

The saliva is often spoken of as containing an anticoagulin to prevent the proteins of the host's blood from coagulating. Actually such a function seems necessary because the blood is stored in the ventral reservoir for a considerable time before it is received into the stomach for digestion. Its function may also consist in dilating the host's dermal capillaries and thus making the blood flow more freely. Kulagin (1905) makes an amusing suggestion which cannot be taken seriously. He says, "probably our blood is too thick and the fly thins it a little with his fluid". When the large amount of blood sucked by the mosquito is compared with the very small amount of fluid which the salivary glands could secrete during feeding, it is realized that no appreciable physical dilution takes place.

The role of the saliva might be thought to consist in preventing the red blood corpuscles from agglutinating and clogging the labral canal. Shute (1935) finds that the blood of man and many mammals when put on a slide with dissected glands of *Anopheles maculipennis* shows complete agglutination. The glands of several other genera of mosquitoes, Shute finds, do not cause agglutination. As yet it is difficult to appreciate the significance of these facts.

The maxillae

From the short historical survey it is seen that the work performed by the maxillae has never been underestimated, and that the earliest investigators assigned to them the chief part in biting. Thus Réaumur (1734) says they penetrate first, clearing the way for the other stylets. Dimmock (1881) says their function is "probably to draw the other mouthparts into the skin". Blanchard (1905) agrees with Réaumur. On reading through the extensive literature on the subject, one finds that almost all later authors assume, and with good cause, the importance of these stylets to the insect.

The movements of the maxilla come about by means of the single protractor and the dorsal and ventral retractor muscles which work exclusively on the stipes. The movement is transmitted to the cutting blade (galea) by means of the galeo-stipital joint where the external galea articulates with the internal stipes. This transmission is possible only by virtue of the soft membranous nature of the ventral part of the rostrum which stretches and bends as the maxilla is protracted and retracted. If the maxilla is worked while the other elements of the proboscis are at rest, the labium too is affected and makes corresponding movements. This is because the galeo-stipital joints are situated close to the basal corners of the theca and also because the dorsal retractor muscles send a few small fibres to these corners. This has no significance in the piercing mechanism, but well illustrates the soft structure of the rostrum in this region, which is a necessity if maxillary movements are to take place. These "resting" movements of the maxilla can be observed in mosquitoes which are coming out of anaesthesia.

During the experiments it was always found to be easy to part the complete fascicle by means of needles from its sheath, the labial theca. In other words, the stylets adhered strongly to one another in a distinct and tight bundle which could be scooped out of its sheath without fear of its splaying into its separate components. This was very striking, and it soon became apparent that this compactness was brought about by the agency of a viscous liquid, always present in the labial groove, which held the mouthparts together by surface tension. A very small spot of olive oil on the end of a needle was used to anoint the fascicle, and with the consequent release of surface tension the stylets could be easily sorted out. In using the olive oil care had to be taken that it did not creep over the body of the mosquito and so put the spiracles out of action. The liquid embracing the mouthparts has use in their lubrication, keeping them supple and preventing their clogging by dried blood, and it will be maintained below that it also has high importance in the piercing mechanism.

In deficiency experiments the particular stylets under consideration were snapped off with a small mounted needle. After the insects had recovered from the anaesthetic they would immediately start cleaning themselves with their front legs in the manner commonly seen in flies and other insects. In this

way they cleared themselves of surplus olive oil, and after raising their palps re-ensheathed their stylets, still using their front legs. Nevertheless Macgregor (1931) attributes re-ensheathment to "an intimate co-operative movement between the proboscis and the labium of a superimposed rotary type".

Many insects were deprived of one galea, and then, after a period of recovery, put with the rabbit for one or two hours a day. Sometimes they were offered the observer's hand from which to feed. It was found that whereas just over half the flies never fed again and, therefore, soon died, the remaining number fed as usual, and continued living normal lives. When one takes a number of mosquitoes from their community in order to give them a feed, certain of them will not draw blood; these are apparently getting old and losing their desire to take food. In galeal deficiency experiments it was not always possible to use young females, and a proportion of the refusers certainly belonged to the category just mentioned, viz. "those too old to feed". Others, no doubt, were slightly injured during the operation. It can, therefore, be said with fairness that a healthy mosquito is able to feed when deprived of one maxillary stylet, but it is observed that more difficulty is encountered than with the full equipment of mouthparts. It is a remarkable fact that this insect is not more seriously affected by the loss of a galea, and it seems very likely that Roubaud has overestimated the difference in habit of the insect that would accrue from a difference in maxillary index. If a mosquito can still feed fairly well with one galea entirely missing, would the addition or subtraction of a tooth on the stylets make all the difference between feeding and not feeding in a crowded community? It would also be interesting to know whether or not the maxillary index is inherited in a Mendelian manner.

A few insects were deprived of both galeae, and these could not be induced to feed. In one case there was doubt as to whether one individual had fed or not, but in any case more results which are positive would have to be obtained before one could generalize about the penetrating ability of mosquitoes lacking both galeae.

These experiments show the function of the maxillae is to help in efficient and smooth penetration of the host's skin, although it is possible for the fascicle to effect a somewhat laboured entry with one galea missing.

The labium

In the early days naturalists noticed that the female mosquito tentatively probed the skin before biting, and they recorded that the labium took no part in penetration but bent into a loop beneath the head while the stylet bundle made the wound. Thus Réaumur (1734) says that the labella are opened and pressed firmly on the skin of the victim; they serve as a support when the insect withdraws its sting. The labium bends back beneath the head as the setae enter more deeply into the skin. Little more is added to this by later authors such as Dimmock (1881), Nuttall & Shipley (1901), Blanchard (1905), Thompson (1906), Manson-Bahr (1925), and Martini (1930), except that there

is a general insistence that the labella guide the fascicle "as a billiard player guides his cue". Patton & Cragg (1913) and Vogel (1920), however, give notes on the transverse crevices of the theca, which, as stated above, make for easy bending of the organ during biting. Vogel adds a comparison of the mosquito's pseudotracheae with the pads on the underside of a gecko's foot.

At the onset of biting, the theca assumes a slight bend which becomes more and more pronounced until finally it is of the hairpin type, doubled on itself underneath the insect's head. While this is happening the apex of the hairpin tends to travel a little way towards the base of the theca. The most acute bend, therefore, is in the proximal half of the theca which lacks muscles. The kinking of the labium can thus be attributed only to indirect muscular action. Describing *Culex*, Vogel (1920) says that the labium bends by means of "**V**-shaped muscle fibres". However this is unlikely for two reasons: first, these muscles are in the distal half of the theca where least bending takes place, and, secondly, these muscles are absent in *Anopheles* where similar movements take place. How then does the labium bend? If the theca is cut across so that only a stump about $\frac{3}{4}$ mm. long is left, that stump will generally be seen to wave backwards and forwards about its base. This movement can be brought about only by the maxillo-labial muscles which act like ordinary flexors in the leg of an insect, for example. Also, if a mosquito be grasped by its wings in mid-air, varying degrees of flexion of the theca may often be observed. Thus kinking of the labium during biting is initiated and probably completed because its proximal part is flexed beneath the head by the paired maxillo-labial muscles. Perhaps pressure from the head may help to some extent, but the statement of Patton & Evans (1929) that "the labium bends passively" cannot be agreed to if it means that pressure from the head is the sole cause of kinking. Thompson (1906) and Vogel (1920) both agree that after the setae have been withdrawn the theca regains its straight shape by its own elasticity. This is easily proved by simple manipulation.

The guiding function of the labella has been very much overestimated by most authors. If the fascicle were a loose bundle of very springy stylets which, with little disturbance, would splay apart (an impression gained from examination of the proboscis of an insect preserved in alcohol), then the labella would subserve the useful role of consolidating the fascicle at its point of impact with the host's skin. But we have seen that the stylets form a tight and compact bundle held together by a viscous liquid, and a guide for them is superfluous. Similarly overrated is the ability of the labella to serve as a point of support for the insect when it withdraws from the host. That these two uses of the labella are unimportant if not actually non-existent can be spectacularly proved by cutting the labella off a number of mosquitoes. Some will die through loss of blood, but certain of those which recover will be found to be able to draw their fill of blood from a rabbit quite normally. For corroborative evidence they have actually been seen to do this on the back of the observer's hand. For Vogel's assumption that the pseudotracheae act as adhesive pads

there has not been found any backing, and the labella were not observed to divaricate to any appreciable extent even during biting. The action of the labellar muscles is to raise the labella slightly, and apparently they are tending to become vestigial and useless. Summing up, we can say that the fascicle is a complete and independent piercing unit capable of working efficiently by itself. The importance of the theca lies in its protection of the fascicle and especially in conserving the fascicular fluid and preventing it from drying up. The labella always grasp the fascicle and during piercing slide up it towards the head; their function is to allow instant return of the stylets to the labial groove after withdrawal and to keep them there in between mealtimes.

The piercing mechanism

The preceding anatomical and experimental studies give a considerable insight into the method used by the female mosquito for insinuating its stylets into the host's skin. While proceeding with the direct story, let us, at the same time, look at one or two remarks of other investigators. For instance, Patton & Cragg (1913) in their *Textbook of Medical Entomology*: "the motive force by which the puncture is made is usually ascribed somewhat vaguely to the forward thrust of the body. To put this in more precise terms would be to assert that the muscles of the legs thrust the body forward, and that this force is conveyed to the piercing stylets through the neck...the neck is anatomically incapable of conveying such a thrusting force, being in most cases entirely membranous...the making of the wound is, therefore, to be regarded as a function of the appendages acting by their own muscles...." These authors evidently think that the words "appendages acting by their own muscles" relieves the neck of responsibility. If the thrust of the maxilla is to make a puncture, the origin area of the maxillary protractor muscle (anterior arm of the tentorium) must be kept more or less stationary by an equal and opposite force to that required to be exerted by the contracting muscle. The neck, Patton & Cragg say, cannot take this strain and we have seen above that the labium is a passive participant. The only alternative is for Patton & Cragg to declare that the equal and opposite force is supplied by gravity acting on the head of the insect. Needless to say, they omit to do this, possibly remembering that the mosquito can make a puncture without necessarily pointing its proboscis towards the centre of the earth. Of the "in most cases entirely membranous" neck it must be said that this organ gives passage to the oesophagus, salivary ducts, nerve cord, two tracheae and muscles which move the head, and that it very efficiently supports the head and proboscis of the insect even under the strain of flying. Blood pressure causes a degree of turgor in the neck, and this would do much to make the organ a firm base for the head. It becomes clear from the above reasoning that the neck most emphatically does "convey the thrusting force" exerted on the thorax by the clinging action of the legs for the purpose of steadying the origin area of the maxillary protractor muscles.

Patton & Cragg continue: "the shape and thinness of the stylets does not render them as liable to fracture as one might suppose for they possess a certain amount of flexibility, probably sufficient to enable them to bend if they meet with any resistant tissue when they are thrust in." The maxillary stylet tapers along its 2·5 mm. length from a width of 0·025 mm. at its base to 0·020 mm. just behind the denticles. It is, therefore, over 100 times longer than the average width of its blade, and over 400 times longer than the diameter of the galeal shaft which is the mainstay of that blade. It will thus be realized that a chitinous bristle of these dimensions will certainly be easily bent, as Patton & Cragg say, but such flexibility is a hindrance rather than a help.

How can the pointed end of such a flimsy stylet enter the skin of the host by means of a muscular thrust at its upper end? Martini (1930) is the only author who attempts to answer this question. He postulates that the bending of the maxillary galea during piercing is prevented by the labial sheath. First, great flexibility is a characteristic of the labium and the cuticle of the labial groove is particularly fine. Secondly, at the onset of penetration the labial sheath loops back and no longer surrounds the fascicle. Thirdly, it has been recorded above that the mosquito can pierce when the labium has been removed, so for these reasons it cannot be agreed that Martini's explanation is valid. The solution to the problem offered here entails a consideration of the viscous liquid in the labial groove. The origin of this liquid is at the moment undetermined, but it seems probable that there is a constant supply of it to cope with losses which might occur in various ways. Therefore, presumably, it is secreted by cuticular glands in the theca, although other sources such as the salivary glands must not be overlooked.

On account of its tubular form the labrum has strength and power of resistance to bending which the other stylets lack. It has been seen that its basal muscles can throw it into a slight sigmoid curve, but it is not within the mosquito's power to bend it any more than this, and still less to make it kink like the labium. It has been seen how the other five stylets cling to the labrum by virtue of the binding effect of the fascicular or labial fluid so that a tight and compact bundle is constituted. The fascicular fluid has high importance for the insect because the maxillary stylets cannot bend to any extent in such a tight bundle. Imagine a perfectly clean and dry fascicle with its point just touching the host's skin; the effect of a maxillary thrust would be merely to bend the galea outwards. Unlike the stylets of some of the plant-bugs (Hemiptera) those of the mosquito are not interlocked along their length in a tongued-and-grooved fashion. Instead, the fascicular fluid, by means of its surface tension, causes the mandibles, hypopharynx and maxillae to cling tightly to the tubular labrum.

The forces at work during the initial maxillary thrust may be summarized as follows:

(1) The maxillary protractor muscle contracts.

(2) Impact with the host's skin causes the galea to experience a bending force tending to part it from the labrum.

(3) The labrum stands firmly enough to withstand any tendency to bend or move sideways.

(4) The fascicular liquid counteracts the bending force experienced by the galea as it binds the latter to the labrum.

(5) The maxillary thrust is propagated undiminished to the tip of the galea which now enters the host's skin.

Both maxillae perform these movements a few times. So much for the initial piercing.

The points of all the stylets are gathered close together by the agency of the fascicular fluid, so that both maxillae co-operate to form one and the same incision which is to be the entry point of labrum, mandibles and hypopharynx. Since the latter stylets move as a whole and function mainly in feeding, they may be conveniently called the "trophic unit" as distinguished from the maxillae which are entirely piercing organs. Now eventually the stylets are thrust into the host's skin for nearly their whole length (commonly about three-quarters of their length), whereas the ambit of action of the protractor muscles of the maxillae is relatively small. Therefore a repetition of small thrusts would have to take place for the attainment of complete penetration, and the mosquito follows up each protraction of the maxillae with a movement of its head nearer to the victim's skin. (Evidence that the maxillae do make quick to-and-fro movements during penetration is afforded by the alternate vibrations of the maxillary palps. At this time these are raised and their elevator muscles originate on the stipites any movement of which is bound to be transmitted to the palps. Movements of the galeae may also be detected through the microscope.) Although such a repetition might require complicated co-ordination of movement of the insect, this course of events is inevitable, because in the mosquito there is no "spare loop of stylets" as in many Hemiptera.

Most authors tacitly convey to the reader that no special events meriting mention take place after the initial puncture, apart from a looping back of the theca. There are two exceptions, however, separated by half a century (Dimmock, 1881; Martini, 1930). The former says that the function of the maxillae is to "draw the other mouthparts into the skin". The stylets "enter the skin as if drawn from below with a slow gliding motion". Martini is a little more explicit when he subscribes to Dimmock's notion: "therefore the maxilla of one side is first thrust into the skin, and with its denticles moored it serves as an abutment and the other is thrust forward further. Then would the latter act as a support and the first could be brought deeper." No evidence, other than the common knowledge that the galeae do possess backwardly pointing teeth, is given by these observers in support of their theory that the maxillae act as grappling hooks. Direct evidence would be very difficult to obtain. Deficiency experiments similar to those described above are incon-

clusive because, as will now be realized, biting entails two distinct phases, viz. puncturing of the epidermis of the host and penetration into the dermis. Thus an experimental mosquito might be capable of performing one phase only, and would, therefore, give a negative result the cause of which could not be safely attributed to failure in one phase or the other. Results could no doubt be obtained from mosquitoes which had been deprived of their galeal teeth but not of their galeal points; the technique required for this experiment would be extremely difficult. A significant point, however, is the well-developed state of the two retractor muscles of each maxilla. Together their strength is approximately double that of the single protractor muscle, and this would hardly be needed if their function were merely to withdraw the stylets after feeding. The galeal teeth would not assist in the puncturing phase, and the grappling hook theory in the second phase provides sufficient cause for their high development. With this admittedly circumstantial evidence, the forces at work during the phase of penetration may be summarized as follows:

(1) The left maxillary protractor muscle contracts.

(2) Any resistance offered by the host's dermis causes the galea to experience a bending force tending to part it from the labrum, and, as in the first phase of biting, this force is counteracted by the surface-tension of the fascicular liquid.

(3) The maxillary thrust is propagated undiminished to the tip of the galea which, therefore, penetrates into the dermis.

(4) The left maxillary protractor relaxes, and left dorsal and ventral retractors contract simultaneously with the right maxillary protractor. Since the left galea is "moored", as Martini describes it, its retractor muscles have the effect of bringing the cranium nearer the host's skin and, therefore, all the other stylets more deeply into the wound. At the same time, the right galea enters more deeply still by protraction.

(5) The right maxillary protractor relaxes, and its dorsal and ventral retractors and the left protractor contract simultaneously, and produce a result similar to that in (4) above.

Quick repetition of this series of movements brings the fascicle to the desired depth in about 20 sec. in man. It is probable that the grip of the legs helps to steady the insect, because mosquitoes which had lost three or four legs were often seen to have difficulty in piercing.

The third phase is one of rest for the mouthparts while the sucking of blood takes place. Kulagin (1905) says that the wound is widened by mandibles and maxillae which perform a sawing action throughout the process of sucking. After complete penetration the maxillae may be needed to cut into a blood vessel, but in several cases the palps were seen to relax and rest on the hand of the observer and the mouthparts to cease to move. Once the insect has started sucking, it seems somewhat comatose so far as external stimuli are concerned. Even cutting of the labium does not curtail feeding activities and only great disturbance makes the insect fly off.

The last phase, that of withdrawal, takes place after the mosquito has taken its fill of blood. Alternating movements of the maxillae again take place, and the fascicle is progressively pulled out with comparative ease and quickness. It has been seen that the labium in no way assists the fascicle during the whole performance of biting. Dimmock (1881), describing withdrawal, says, "when the mosquito withdraws the setae it probably first withdraws the barbed maxillae beyond the other setae, so that the barbs are kept out of action by the mandibles and hypopharynx". This description, although correct, is not very explicit. Continuous pressure exerted by the legs is transmitted through the neck as a lifting force acting on the head and proboscis. First one and then the other maxilla is retracted so that the points of the galeae are behind the points of the other stylets. The maxillary retractors relax and the labrum, mandibles and hypopharynx, obeying the lifting force exerted by the legs, slide up the maxillae until the latter are in the protracted position. (In this way the maxillae, of course, are passively and inversely protracted.) The maxillae are then again retracted in turn, and thus the fascicle is gradually eased out of the wound it has made. It will be recalled that the maxillary galeae taper, and this, together with the fact that the dorsal stylets follow the maxillae into the wound, explains how the returning path of the maxillae is much easier than their path of penetration. Their barbs, now being kept subterminal to the fascicle, are kept as far as possible out of the way of the surrounding tissue. Out of the wound, the fascicle immediately returns to the thecal groove, the palps are lowered and the mosquito flies away.

Summary

The proboscis

1. Vogel's (1920) assertion that the labral canal is closed ventrally by apposition of the edges of the labral groove is fully confirmed. The hypopharynx plays no part in the ventral closure of the food canal except at its base. The somewhat popular term "labrum-epipharynx" is dispensed with in favour of "labrum".

2. The mandibular teeth vary in number round about 40. The mandibular suspension is fully described.

3. Evidence from musculature is put forward for the existence of a small maxillary palpifer not present in other Diptera.

4. Snodgrass (1935) criticizes Crampton's (1925) homologizing of the labella with labial palps because "the lobes of the fly labium have usually each only one muscle inserted directly upon it", whereas palpi usually have antagonistic muscles. Vogel for *Culex* and this paper for *Anopheles maculipennis* show that the labial lobes of these insects have antagonistic muscles like typical palpi.

The piercing mechanism

Phase I. Puncturing the epidermis.

1. The insect alights, brings its proboscis approximately at right angles with the host's skin, raises its palps, probes a few times in preliminary inspection and commences to bite.

2. The mosquito steadies its head, preparatorily to biting, by clinging to the host with its legs, the resultant thrust being transmitted to the head by the slender neck.

3. The maxillo-labial muscles begin to contract and cause the theca to assume a slight bend.

4. The maxillary protractor muscles contract alternately, and the movement is passed on by way of the stipites and palpal elevators to the palps which therefore begin to quiver.

5. Bending of the galeal shaft is prevented by the joint action of the labrum and fascicular fluid. The latter binds the galeae by means of surface tension to the labrum which can resist undue bending on account of its tubular structure.

6. The tips of the galeae receive the muscular thrusts undiminished, and therefore enter the host's epidermis.

Phase II. Penetrating the dermis (a longer process than Phase I).

1. Throughout this phase the maxillo-labial muscles perform progressive contraction so that the theca is looped back beneath the head which is thus not hampered in getting close to the host's skin as the fascicle sinks deeper.

2. The mosquito still clings to the host.

3. The maxillary protractor of one side contracts simultaneously with the two retractors of the opposite side, and vice versa. These alternating maxillary movements are passed on to the palps which wave up and down in turn so long as penetration endures.

4. Still under the stiffening influence of the labrum and fascicular liquid, the galeae enter more deeply during protraction. During retraction, when the galeal teeth hook on to the dermal connective tissue fibres and prevent the stylet from being drawn out of the wound, the head is pulled nearer the host's skin. Therefore the trophic unit is drawn farther into the wound by the action of the retracting maxilla.

5. The terminal sense organs of the labrum serve as directors of the course that the fascicle shall take. The fascicular liquid, being of an oily consistency, still possesses surface tension in the host's lymph spaces, and the compact stylet bundle can, therefore, be steered without difficulty by the labral retractor and elevator muscles.

Phase III. Feeding (the longest phase of all).

1. The fascicle having been steered to a suitable venule which may be dilated under the influence of the insect's saliva, the maxillary galeae make an incision in the vessel.

2. The stylet bundle protrudes into the lumen of the venule, the mandibular retractor muscles withdraw the mandibular blades from the opening of the food canal, and sucking begins.

3. The palpal elevators relax, and the palps rest on the host's skin.

Phase IV. Withdrawal.

1. The mosquito braces itself against the host's skin with its legs, and raises its palps.

2. The maxillary retractors alternately draw the galeae back so that their points are subterminal to the rest of the fascicle.

3. The maxillary retractors relax, and the trophic unit, obeying the lifting force exerted by the legs, slides up the maxillae which are then again retracted, and so the process continues until the proboscis is eased out of the wound.

4. Meanwhile the relaxed labial theca is gradually straightening out under its own elasticity. When the fascicle is completely withdrawn, the theca has assumed its normal extended shape and re-ensheathment automatically ensues. The palps are lowered and the insect flies off.

ACKNOWLEDGEMENTS

Dr Grove has given me much useful advice which was indispensable for the carrying through of the research.

Thanks are due to Mr P. G. Shute of Horton Hospital, Epsom, for supplies of mosquitoes and for very useful directions concerning their maintenance throughout the year in the laboratory.

I am much indebted to Prof. F. E. Fritsch for the use of his heated greenhouse, to Prof. E. H. Lamb for the use of his photomicrographic apparatus, and to Dr F. M. Haines for making a film of the insect biting.

REFERENCES

BARRAUD, P. J. (1934). A practical entomological course for students of malariology. *Government of India Health Bulletin*, no. 18, Malaria Bureau, no. 9.

BECHER, E. (1882). Zur Kenntnis der Mundteile der Dipteren. *Denkschr. Akad. Wiss. Wien*, 45.

BLANCHARD, R. (1905). *Les Moustiques*, Paris.

CHRISTOPHERS, S. R. (1933). *The Fauna of British India*. London.

CORNWALL, J. W. (1923). On the structure of the salivary pump in certain blood-sucking and other insects. *Ind. J. med. Res.* x.

*CRAMPTON, G. C. (1925). A phylogenetic study of the labium of holometabolous insects, with particular reference to the Diptera. *Proc. ent. Soc. Wash.* **27**, 4.

DIMMOCK, G. (1881). *The Anatomy of the Mouthparts and of the Sucking Apparatus of some Diptera*. Boston.

FÜLLEBORN, F. (1908). Über Versuche an Hundefilarien und deren Übertragung durch Mücken. *Arch. Schiffs- u. Trop. Hyg.* **12**, 8.

IMMS, A. D. (1934). *A General Textbook of Entomology*. London.

*JOBLING, B. (1928). The structure of the head and mouthparts in *Culicoides pulicaris* L. *Bull. ent. Res.* **18**.

KELLOGG, V. L. (1899). The mouthparts of the nematocerous Diptera. *Psyche*, **8**.

*KRÄPELIN, K. (1882). Über die Mundwerksäuge der saugenden Insecten. *Zool. Anz.* **5**.

KULAGIN, N. (1905). Der Kopfbau bei *Culex* und *Anopheles*. *Z. wiss. Zool.* **83**.

LÉON, N. (1904). Vorläufige Mitteilung über den Saugrüssel der Anopheliden. *Zool. Anz.* **27**.

E. Martini.

MANSON-BAHR, P. (1925). *A Handbook of Tropical Diseases.*

MACCLOSKIE, G. (1888). The poison apparatus of the mosquito. *Amer. Nat.* **22**.

MACGREGOR, M. E. (1931). The nutrition of adult mosquitoes. *Trans. R. Soc. trop. Med. Hyg.* **24**.

MARTINI, E. (1930). *Culicidae*, in Lindner's *Die Fliegen der palaearktischen Region*, 11 and 12. Stuttgart.

NUTTALL, G. F. & SHIPLEY, A. E. (1901). The structure and biology of *Anopheles maculipennis*. *J. Hyg., Camb.*, **1**.

—— —— (1903.) The structure and biology of *Anopheles maculipennis*. *J. Hyg., Camb.*, **3**.

PATTON, W. S. & CRAGG, F. W. (1913). *A Textbook of Medical Entomology.* London, Madras and Calcutta.

PATTON, W. S. & EVANS, A. M. (1929). *Insects, Ticks, Mites and Venomous Animals of Medical and Veterinary Importance.* Liverpool Sch. Trop. Med.

*PETERSON, A. (1916). The head-capsule and mouthparts of Diptera. *Illinois biol. Monog.* **3**, 2.

RÉAUMUR, M. (1734). *Mémoires pour servir à l'histoire des Insectes*, **4**. Paris.

ROBINSON, G. G. (1938). The head-capsule, the mouthparts and their musculature in the female mosquito, *Anopheles maculipennis*. Thesis for M.Sc., University of London.

ROUBAUD, E. (1928). Nouvelles recherches sur l'évolution zoöphile des faunes d'*Anopheles* en Europe. *Ann. Inst. Pasteur.* **42**.

SHUTE, P. G. (1935). Agglutination of the red blood-corpuscles of man, animals and birds by the salivary glands of *Anopheles maculipennis*. *J. trop. Med. Hyg.* **38**.

—— (1936). A simple method of rearing and maintaining *Anopheles maculipennis* throughout the year in the laboratory. *J. trop. Med. Hyg.* **39**.

SMITH, J. B. (1890). A contribution to a knowledge of the mouthparts of the Diptera. *Trans. Amer. ent. Soc.* **17**.

SNODGRASS, R. E. (1935). *The Principles of Insect Morphology.* New York and London.

THEOBALD, F. V. (1901–3). *A Monograph of Culicidae or Mosquitoes*, **1–3**. London.

THOMPSON, M. T. (1906). The alimentary canal of the mosquito. *Proc. Bost. Soc. nat. Hist.* **32**.

VOGEL, R. (1920). Kritische und ergänzende Mitteilungen zur Anatomie des Stechsapparats der Culiciden und Tabaniden. *Zool. J.* **42**.

* No direct reference to mosquitoes.

NOTES ON SOME NEW SPECIES OF PARASITIC MITES

By CHARLES D. RADFORD

(With 15 Figures in the Text)

Introduction

Examination of mite material sent from various sources for identification has revealed a number of new species. In this paper seven new species of Laelaptinae, one of *Entonyssus* and one of *Listrophoroides* are described.

The genus *Cyclolaelaps* was erected by Ewing (1933), with *C. circularis* as the type, for those Laelaptinae in which the body was "almost circular and not thickly beset with hairs...genito-ventral plate with a single pair of hairs... legs short and stout". Mites which appear to belong to this genus, *Cyclolaelaps*, were received from Mr G. H. E. Hopkins, Entomologist, Kampala, Uganda, having been collected on a mouse (*Lophuromys aquilus aquilus* True). Similar mites have been taken on the black rat (*Rattus rattus* L.).

Dr Flavio da Fonseca (1935) erected a number of new genera of Laelaptinae. Among them was the new genus *Ischnolaelaps* for Laelaptinae which are elliptical, poorly chitinized, have the genito-ventral shield slightly expanded posteriorly and have thereon only the genital pair of spines.

From the South African Zoological Survey Committee, through P. J. du Toit, Esq., Director of Veterinary Services, Onderstepoort, I have received a number of tubes containing mites from various rodents. Amongst this material there are three new species of this genus *Ischnolaelaps*, and a new species of *Listrophoroides*. The other three species of *Ischnolaelaps* were sent from Kampala.

From Brevet-Col. A. E. Hamerton, C.M.G., D.S.O., Pathologist, Zoological Society of London, I received the lungs of a North American garter snake (*Thamnophis sirtalis*), containing one male and two females of a new species of *Entonyssus*.

To the above gentlemen I wish to acknowledge my indebtedness for the material.

Genus **Cyclolaelaps** Ewing, 1933

Cyclolaelaps lophuromius n.sp.

The ventral surface of the female (Fig. 1) shows the sternal shield to be almost square, the anterior border slightly convex, and the posterior edge somewhat concave. The shield bears the usual three pairs of spines and in

addition has two pairs of slits. Close to coxae iii and iv there is an accessory plate bearing a spine similar to those of the sternal shield.

On the genito-ventral shield there is only the genital pair of spines, with three pairs of similar spines bordering it as shown. The anal shield is broadly rounded in front and bluntly pointed at the posterior end. The anal pore lies in the anterior half of the shield with the paired anal setae slightly behind its median line. The third anal spine lies about half-way between the anal pore and the end of the shield, just in front of the pitted area.

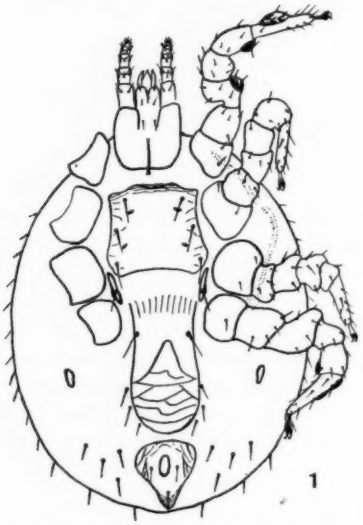

Fig. 1. *Cyclolaelaps lophuromius*. Venter of female.

Behind the fourth pair of legs and almost midway between the genito-ventral shield and lateral edge of the body there is a pair of metapodal shields. In addition to the spines already mentioned there are four pairs of spines arranged round the anal shield as figured, whilst around the edge of the body there are several pairs of spines.

The legs are short and stout and bear several areas on them which are strongly chitinized. The stigmal pore is situated between coxae iii and iv with the peritreme running in a sinuous way to the middle of coxa i.

The dorsal shield appears to be without sculpturing, is broadly oval, covering the dorsal surface from its anterior margin to the level of the second pair of legs. From thence it does not cover the dorsum but leaves an exposed border around it.

Host. Mouse (*Lophuromys aquilus aquilus* True).

Locality. Kampala, Uganda, 31 August 1937.

Measurements. ♀, 0·61 × 0·49 mm. (without capitulum).

Genus **Ischnolaelaps** Fonseca, 1935

Ischnolaelaps dasymys n.sp.

The venter of the female (Fig. 2) shows the sternal shield to be placed between coxa ii and slightly backwards to coxa iii. It bears the usual three pairs of spines and the two pairs of slits or pores which are present in this genus (as also in Ewing's genus *Cyclolaelaps*). The anterior edge of this shield

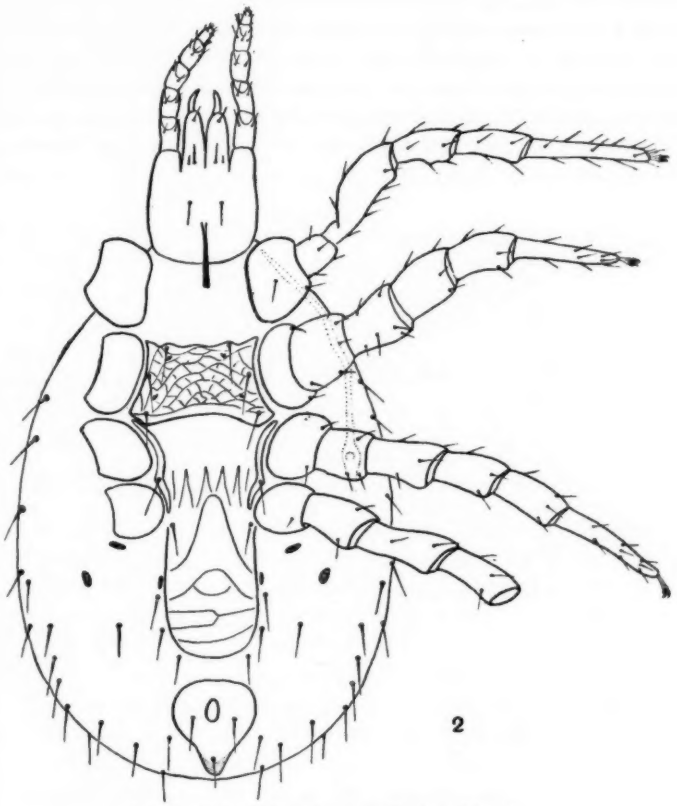

Fig. 2. *Ischnolaelaps dasymys*. Venter of female.

is emarginate and the posterior edge concave. Extending from the postero-lateral corners of the sternal shield to the middle of coxa iv are the metasternal plates, each bearing a single spine.

The genito-ventral shield extends backwards to about midway between coxa iv and the posterior end of the body, and bears the single pair of genital spines. Bordering this shield are three pairs of spines with a fourth pair placed further out towards the lateral border of the body.

The anal shield is rounded anteriorly, pear-shaped, with the pore placed in the anterior half. The paired anal spines are placed on a level with the posterior end of the pore and about midway between it and the edge of the shield. The unpaired spine is placed in the middle of the posterior half.

In addition there are three pairs of metapodal plates, shaped and placed as shown, together with a number of spines on the venter proper with several round the periphery.

The legs are long and slender, particularly the first and fourth whilst the second pair of legs are somewhat stouter. The dorsal shield covers the entire dorsal surface and is, like the sternal shield, reticulated.

Host. East African swamp rat (*Dasymys helukus helukus* Heller).

Locality. Kakumiro, Uganda, 5 October 1937.

Measurement. ♀, 0·83 × 0·6 mm. (without capitulum).

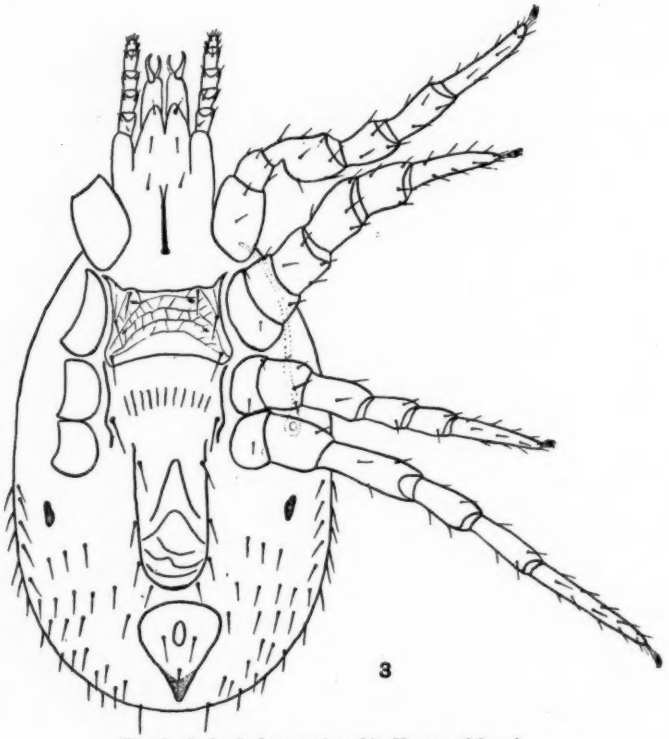

Fig. 3. *Ischnolaelaps arvicanthis.* Venter of female.

Ischnolaelaps arvicanthis n.sp.

The venter of the female (Fig. 3) shows the sternal shield to be furnished with the usual three pairs of spines and the two pairs of pores also, the reticula-

tions as in the previous species. The anterior corners of the shield project a short distance round coxa ii, whilst the posterior corners are more rounded. The metasternal plates are not so strongly chitinized, only the outer edge being discernible. The genito-ventral shield is almost straight-sided, possesses the single pair of genital spines, with the three additional pairs of spines round the edge.

The anal shield, rounded anteriorly is produced posteriorly to a blunt point. The pore is placed close to the centre of the shield with the paired setae just before its posterior end, and the unpaired seta is placed in the middle of the posterior half of the shield. The metapodal shields are irregularly oval and placed well out towards the lateral border of the body. Some twenty-five pairs of spines are situated on the soft integument of the venter with a number of spines round the periphery.

Legs somewhat slender, especially leg iv; leg ii short and stout; spines of legs slight.

Dorsal shield reticulated, covering most of the dorsum with the exception of a very narrow strip at the posterior end of the body. Dorsal spines similar to those present on the sternal shield, fairly numerous.

Host. Uganda unstriped grass rat (*Arvicanthis abyssinicus rubescens* Wroughton).

Locality. Kakumiro, Uganda, 9 October 1937; Kitgum, 20 January 1938.

Measurement. ♀, 0·77 × 0·52 mm. (without capitulum).

Ischnolaelaps tateronis n.sp.

On the venter of the female (Fig. 4) it will be seen that the anterior corners of the sternal shield project between the coxae of legs i and ii, the front edge of the shield is almost straight and possesses the usual spines and pores.

The metasternal plates extend from the posterior corners of the sternal shield to coxa iv, with a slight projection between coxae iii and iv. The genito-ventral shield possesses only the genital pair of spines, with two pairs of spines bordering the shield and a third pair between it and the anal shield. The anal shield is elongate pear-shaped, the paired setae are placed just before the posterior end of the pore, which is close to the centre of the shield. The unpaired seta is placed in the middle of the posterior half of the shield.

The metapodal shield is placed well out to the lateral edge of the body and is rod-like. There are about eight pairs of spines on the venter in addition to those already mentioned.

The dorsal shield almost completely covers the dorsum, leaving only a narrow strip round its postero-lateral edge, from the level of the fourth pair of legs.

Host. Gerbille (*Taterona benvenuta* Hinton & Kershaw).

Locality. Toio River, Gulu District, Uganda, 5 December 1937.

Measurement. ♀, 0·53 × 0·35 mm. (without capitulum).

Ischnolaelaps cryptomius n.sp.

The female venter (Fig. 5) shows the sternal shield to be wider than long, with its sides concave and the anterior corners projecting towards coxa i. The posterior corners are rounded and reach almost to the middle of coxa iii. The metasternal plates are short, although they occupy the same position (coxae iii–iv) as in the other species, in this species the coxae are placed closer together.

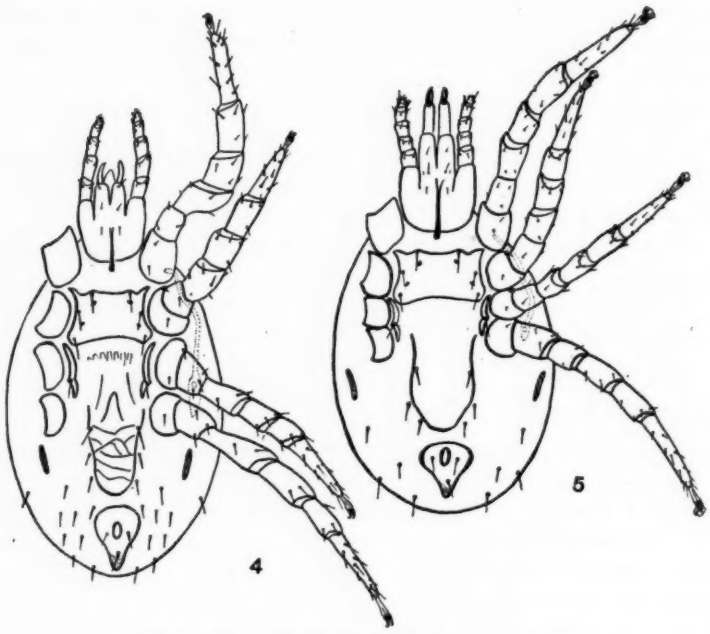

Fig. 4. *Ischnolaelaps tateronis.* Venter of female.
Fig. 5. *Ischnolaelaps cryptomius.* Venter of female.

On the genito-ventral shield there is only the genital pair of spines and two pairs of spines round the border. The anal shield is as wide as long, with the paired setae placed about level with the middle of the pore, which is in the middle of the anterior half of the shield. The unpaired spine is almost in the centre of the posterior half of the shield. The metapodal shield is long and slender and close to the lateral edge of the venter. About four pairs of spines are placed on the soft integument of the ventral surface.

The dorsal shield is oval, covering the entire dorsum from the anterior border to coxa ii, from whence it becomes progressively narrower, leaving a

wider and wider strip of body exposed and does not reach further backwards than just over midway from coxa iv to the posterior end of the body.

Host. Cape, blesmol (*Cryptomys capensis* Cuv.).

Locality. Wynberg, Cape Province, 11 July 1938.

Measurement. ♀, 0·47 × 0·35 mm. (without capitulum).

Ischnolaelaps rhabdomys n.sp.

The female venter (Fig. 6) shows the sternal shield to have an undulating anterior border with the corners projecting between coxae i and ii. The shield is slightly wider than long and is reticulated over most of its area.

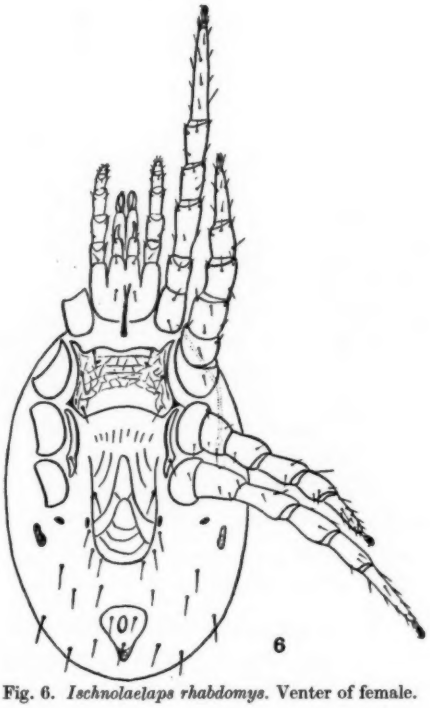

Fig. 6. *Ischnolaelaps rhabdomys.* Venter of female.

The anterior portion of the metasternal plate is longer than the posterior portion and bears the usual single spine. The genito-ventral shield is not long, extending only a short distance behind the fourth pair of legs. It has the single pair of genital spines and is bordered by three pairs of spines. The anal shield is widely separated from the genito-ventral shield and it will be seen that the paired anal spines are placed on a level with the anterior end of the anal pore. There are three pairs of metapodal shields in this species, two lateral and a small oval one close to the genito-ventral shield.

The dorsal shield is oval, covering the anterior part of the dorsum to a level with the second pair of legs, whence it leaves a moderately wide strip of exposed soft integument round its periphery.

Host. Striped mouse (*Rhabdomys pumilio* Sparrm.).

Locality. Onderstepoort, Transvaal, 25 August 1938.

Measurement. ♀, 0·6 × 0·37 mm. (without capitulum).

Ischnolaelaps bathyergus n.sp.

This is the first species of this genus in which males have been found. In the tube were eighteen males and thirty-three females, no other mites being included. In a second tube from the type host, taken at the same site on the

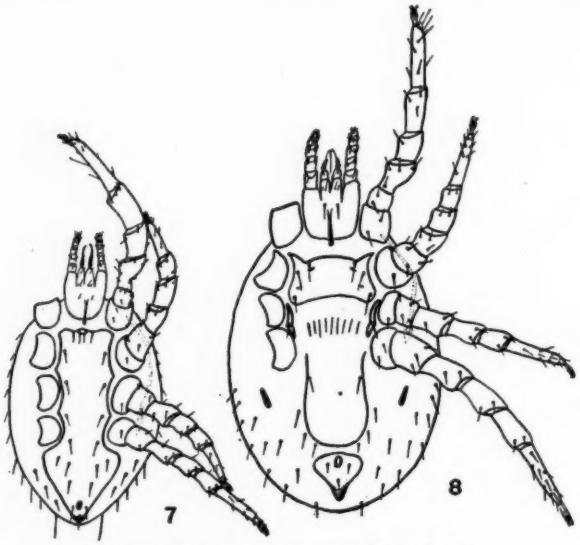

Fig. 7. *Ischnolaelaps bathyergus.* Venter of male.
Fig. 8. *Ischnolaelaps bathyergus.* Venter of female.

same date there were five males and thirty-one females. The presence of these males and the absence of any other mites points conclusively to the fact that they are the males of the species.

On the male venter (Fig. 7) it will be seen that the ventral shields are fused into one and cover the greater portion of the area between the coxae, expanding behind coxa iv, then tapering to a bluntly rounded point close to the posterior end of the body. The shield has nine pairs of spines (including the paired anal spines) and the unpaired anal spine. There are also three pairs of spines on the soft integument of the venter and five pairs round the posterolateral border, the terminal pair being longer and stiffer than the others.

The dorsal shield covers the entire dorsal surface, leaving no exposed integument.

The venter of the female (Fig. 8) shows the sternal shield with the anterior corners projecting between coxae i and ii. The metasternal plates are small, the anterior portion being longer than the posterior. The genito-ventral shield is expanded behind the genital pair of spines, and has the three pairs of spines round its edge. The anterior angles of the anal shield are wide and the shield is deeply concaved laterally. The pore is placed well forward on the shield with the paired setae level with its posterior end. Midway between the genito-ventral shield and the lateral border of the body is a long oval metapodal shield.

The soft integument of the venter is furnished with eleven pairs of spines, as shown. The dorsal shield covers the entire dorsum.

Host. Cape dune mole (*Bathyergus suillus* Schreb.).

Locality. Strandfontein, Cape Town, 7 April 1938.

Measurement. ♂, 0·34 × 0·23 mm. ♀, 0·47 × 0·31 mm. (without capitulum).

Genus **Entonyssus** Ewing, 1922

Entonyssus hamertoni n.sp.

The male venter (Fig. 9) shows a portion of the sterno-genital shield (the weak chitinization of the shields in these snake lung mites makes it very difficult accurately to define the outline). The genital pore is seen to be placed in the middle of the anterior edge of the shield. There are five pairs of spines down the middle of the venter and the anal shield is placed at the extreme tip of the body, with a portion showing on the dorsal aspect. The dorsal shield does not cover much of the dorsum, leaving a wide margin of exposed integument.

The mandibles of the male are shown in Fig. 12, whilst the tarsus of leg i shows the peculiar eye spots present on the tarsal sucker. This is a very noticeable feature in this species and is present in both sexes, but only on the tarsi of the first pair of legs (Fig. 11).

The venter of the female (Fig. 10) shows the general shape and size to be similar to that of the male. The sternal shield extends from coxa ii to the middle of coxa iii, with lateral projections between coxae i and ii, and between ii and iii, and is furnished with three pairs of spines. The genito-ventral shield is provided with the single pair of genital spines, and between this and the anal shield is a pair of spines. The paired anal spines are placed level with the anterior end of the anal pore, and the unpaired seta is placed at the extreme tip of the body. The mandible of the female (Fig. 13) shows the fixed arm to possess on its inner edge a single strong tooth.

Host. North American garter snake (*Thamnophis sirtalis*).

Locality. Zoological Gardens, Regent's Park, London, 2 December 1938.

Measurement. ♂, 0·5 × 0·3 mm. ♀, 0·5 × 0·3 mm. (without capitulum).

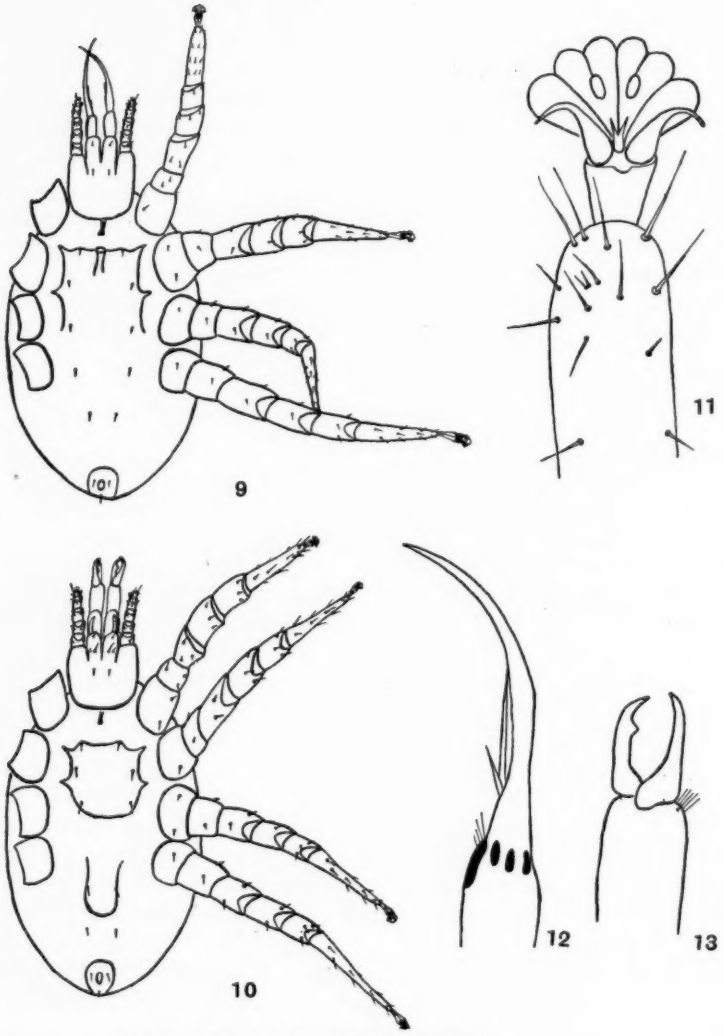

Fig. 9. *Entonyssus hamertoni*. Venter of male.
Fig. 10. *Entonyssus hamertoni*. Venter of female.
Fig. 11. *Entonyssus hamertoni*. Tarsus i, female.
Fig. 12. *Entonyssus hamertoni*. Right mandible, male.
Fig. 13. *Entonyssus hamertoni*. Right mandible, female.

Genus **Listrophoroides** Hirst, 1923

In this genus the body is flattened dorso-ventrally instead of laterally, as in the genus *Listrophorus*. All tarsi are provided with a small pulvillus. Terminal segments of legs i and ii grooved ventrally, the sides of the grooves provided with processes for grasping the hairs of the host.

Listrophoroides bathyergians n.sp.

The venter of the male (Fig. 14) shows the large area of the chitinized shields present in the genus. The hairs on the venter are very small and in some specimens almost invisible—one pair on the capitulum, one between legs i and ii, four pairs round the penis, which is a small hook-like organ lying between

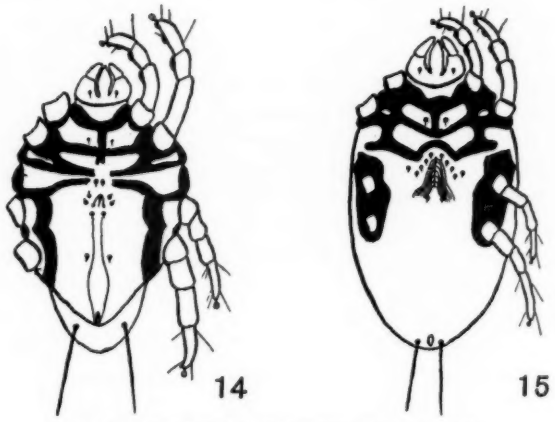

Fig. 14. *Listrophoroides bathyergians*. Venter of male.
Fig. 15. *Listrophoroides bathyergians*. Venter of female.

coxae iii, and a further pair of similar hairs between coxae iv. The anal pore is placed some little distance from the posterior end of the body and slightly behind it rises a pair of long and stiff bristles. The legs appear to be practically devoid of hairs, except the tarsi which have two or three pairs.

The venter of the female (Fig. 15) shows a different pattern of the chitinized shields. There are the small hairs on the capitulum and between coxae i and ii, as in the male, but in the region of the genital pore the five pairs of minute hairs are arranged as shown. Behind the genital pore is a further pair of small hairs. Coxae iii and iv are situated ventrally on a pair of chitinized shields, and the posterior end of the body is terminated by a pair of long, stiff bristles. The third and fourth pairs of legs are weaker in the female than in the male, where in the latter the last pair of legs is stouter than the remainder.

Host. Cape dune mole (*Bathyergus suillus* Schreb.).

Locality. Strandfontein, Cape Town, 7 April 1938.

Measurement. ♂, 0·34 × 0·2 mm. ♀, 0·37 × 0·21 mm. (including capitulum).

REFERENCES

EWING, H. E. (1922). *Proc. U.S. Nat. Mus.* **63**, 13, 1–26.
—— (1924). *Proc. ent. Soc. Wash.* **26**.
—— (1933). *Proc. U.S. Nat. Mus.* **82**, 30, 1–14.
FONSECA, F. DA (1935). *Congres Internat. de Zool.* Section 8, Lisbon, pp. 1597–1615.
HIRST, S. (1923). *Proc. zool. Soc. Lond.* pp. 971–1000.
RADFORD, C. D. (1937). *Northw. Nat.* pp. 38–42.
VITZTHUM, H. GRAF (1935). *Z. Parasitenk.* **7**, 709–16.

(*MS. received for publication* 2. II. 1939.—Ed.)

ON "*TERANYMPHA*" AND OTHER MONSTROUS LATIN PARASITES

By CLIFFORD DOBELL, F.R.S.
National Institute for Medical Research, London, N.W. 3

Semper ego auditor tantum? nunquamne reponam?

In a recent paper Dr L. R. Cleveland (1938) has published some observations on a remarkable flagellate called "*Teranympha*", which was discovered nearly 25 years ago by Dr M. Koidzumi in the white ants of Japan. I do not now propose to discuss this singular organism itself, however, but only its name and certain other matters of wider interest which its nomenclature suggests—matters which seem to me, indeed, to merit the immediate attention not only of all parasitologists but even of zoologists generally.

Koidzumi first described and figured his flagellate in the second of two Government Reports (Formosa, 1916, 1917) written in Japanese, and therefore inaccessible and unintelligible to most European and American workers. After the War, however, as he desired to make his protozoological findings generally available, he sought my assistance; and through the generosity of the Medical Research Council he was enabled to work in my laboratory in 1920, and to republish his researches—with their original illustrations—next year in *Parasitology* (Koidzumi, 1921). With my aid he then carefully corrected his Japanese reports and rewrote them in English—the final version, in this language, thus differing considerably from the originals.

In his Japanese report Koidzumi named his remarkable new flagellate *Teranympha mirabilis*; but in the later English version he changed its generic designation to *Teratonympha*. His reason for so doing was given in the following words: "In my original paper in Japanese (1917) I wrote this name as '*Teranympha*'—an orthographic error which I now correct" (Koidzumi, 1921, p. 260). Dr Cleveland (1938, p. 442) now says "It is doubtful if Koidzumi had the right to correct this error in spelling", though he gives no reasons for his doubt: and he then, somewhat illogically, apparently assumes that Koidzumi had *no* right to do so, as he adopts the name "*Teranympha*" instead of *Teratonympha*. It was on my advice, however, that Dr Koidzumi corrected the name of his flagellate; and as he is now less able to defend his action than I am, it is clearly my duty to justify his statement in answer to Dr Cleveland's criticism.

Many recent writers—not only Dr Cleveland—appear to believe that the Rule of Priority prohibits the correction of errors in the formation and spelling

of generic and specific names: but I believe this is merely a misapprehension due to the mistranslation of Article 19 in the English version of the *International Rules of Zoological Nomenclature*. The French version—which is the original, and takes precedence of all others—reads as follows:

"Art. 19.—L'orthographe originelle d'un nom doit être conservée, à moins qu'il ne soit évident que ce nom renferme une faute de transcription, d'orthographe ou d'impression."

Nothing could be more definite: the rule is expressed with all the exactitude possible in French (and equally in English when employed properly). But unfortunately the English translation of this article reads:

"Art. 19. The original orthography of a name is to be preserved unless an error of transcription, a *lapsus calami*, or a typographical error is evident."

I say, without fear of contradiction, that "*lapsus calami*" is not a correct English translation of "*faute d'orthographe*": the translator here not only used the wrong language, but he entirely altered the sense of his prototype. The French rule says nothing about "a slip of the pen", but specifies "*an error in orthography*" as one of the things which it is permissible to correct in a name: indeed, the rule implies that *miswritten words are to be corrected*. As a distinguished French palaeontologist[1] has pointed out, "Il ressort de cet article que la correction des barbarismes ou solécismes d'accord des genres s'impose d'office et doit obligatoirement être faite sans qu'il en résulte une violation de la loi de priorité."

Consequently, I had no hesitation in advising Dr Koidzumi to amend the original spelling of his name "*Teranympha*" (1917) to *Teratonympha* (1921). It is derived from the Greek[2] τέρας, gen. τέρατος [= monster], and the second form is therefore correct: the first is merely an error of word-formation and orthography.

As will be observed, Art. 19 stipulates that a fault which is corrected must be evident in the original: and it might be objected that the derivation of "*Teranympha*" is not "evident". I was informed by Dr Koidzumi himself, however, that he intended to combine *Teras* [monster] with *nympha* (on analogy with *Trichonympha*), and made his mistake through ignorance of Greek and Latin. If it be further objected that this is merely my private information, and not self-evident from his publication, I would point out that he referred to the same organism earlier, in his first Japanese report (1916), as *Metameronympha monstrosa*[3]—which clearly indicates his intention, and the derivation of "*Teranympha*".

[1] M. Cossmann, in a note appended to the reprint of the Rules published in his *Rev. crit. Paléozool.* **18** (1914).

[2] I am aware that some editions of the well-known American *Medical Dictionary* of Dorland state that "*Teras*" is "Latin for a monster"; but this is not, I believe, the opinion of classical scholars.

[3] This name, published in Japanese, without any definition or description, is clearly invalid—as I pointed out to Dr Koidzumi in 1920, and as Dr Cleveland now also notes. It is *nomen nudum*.

Accordingly, the correct name of the metamerically segmented flagellate of Japanese termites would appear to be *Teratonympha mirabilis* Koidzumi, 1917 (emend. 1921), and not "*Teranympha*" (without any specific designation) as Dr Cleveland seemingly supposes.

But unfortunately the nomenclature of this flagellate cannot be decided so simply. It is complicated by the fact—noted by Cleveland—that the same organism was discovered and named independently by Prof. V. Dogiel, at almost the same time, in Russia. Dogiel found *Teratonympha mirabilis* in termites[1] sent to him from Japan by Dr S. Ono in 1916, and described and named it *Cyclonympha strobila* in the following year (Dogiel, 1917)—in ignorance of Koidzumi's work. According to Cleveland, Koidzumi's paper was published in March 1917, and Dogiel's on 2 August 1917: consequently, Koidzumi's name has priority. But it is also unfortunate that Cleveland does not give his evidence for these exact dates. I have been unable myself—after much inquiry—to ascertain the real date of publication of either paper. My own copy of Dogiel's bears the date 1917 alone (no day or month), while even in 1920 Koidzumi himself was unable to determine the precise time of issue of his own memoir.

Moreover, there are two other difficulties connected with the names *Teratonympha* and *Cyclonympha*, to neither of which Cleveland alludes. The first is the language of "publication". Are *all* languages equally legitimate for the publication of new species under the International Code? If Russian and even Japanese are accepted, then why not Basque, Arabic, Marathi, Thibetan, or Swahili? If I were to propose a new species in a paper written in Cornish would it be valid, and have priority over another name introduced subsequently by someone else in a more orthodox zoological language such as French, German, or Italian? This is a question which has never yet—so far as I am aware—been considered and answered by the International Commission on Nomenclature: but it is one to which an answer is clearly desirable.[2] At present it seems that Japanese—a language unreadable by most zoologists—is an acceptable vehicle for the introduction of new names and definitions into zoology: yet it is obvious that adoption of the general principle here involved is likely, some day, to lead to great confusion.

The second difficulty is this: granted that Russian and Japanese are both valid zoological languages, what is the status, under the International Code, of names like *Cyclonympha*—proposed at a time when they cannot be made known generally? For Dogiel's paper was "published" in Russia, in 1917, at the time of the Revolution; and for some years afterwards it was unknown and unknowable to most workers outside Russia owing to the prohibition placed on

[1] Said to be *Coptotermes*, but clearly in error—as Cleveland points out. *Teratonympha* lives only in *Leucotermes* (= *Reticulitermes*), as Koidzumi correctly states.

[2] Appendix A, to the International Rules, does not meet the case. It merely expresses the pious opinion that "it is very desirable" (but not compulsory) that new names should be accompanied by a diagnosis in English, French, German, Italian, or Latin.

the export of all publications by the Bolshevik Government. Does "publication" under such restrictions constitute "publication" within the meaning of the International Code? I do not know: yet I think that the date of "*Cyclonympha* Dogiel, 1917" is not really 1917, but rather the date on which his memoir first became available to the world at large. A name can hardly be regarded as "published" while it is forcibly withheld from the public.

After due consideration, therefore, I conclude that Koidzumi's amended name *Teratonympha* is both correct and valid, and that it should take precedence of *Cyclonympha* Dogiel. But the status of both names is debatable, so I draw this conclusion not without misgivings.

Unhappily, "*Teranympha*" is no isolated instance of the modern misuse of languages in zoology. Scores of similarly malformed names abound everywhere, while false concords between genera and species are to be found in almost every recent book and periodical. (I speak particularly of protozoological publications, though protozoologists are not the only sinners. I also say nothing of hybrids, which are now generally preferred, apparently, to pure Greek or Latin.) To take a few examples at random: I find that the specific name "*termitidis*" (in mistake for *termitis*, the regular Latin genitive of *Termes*) has been given to several "parasites" of white ants;[1] while a new trematode from the beaver (*Castor*, gen. *castoris*) has lately been named *Paramphistomum castori* by Kofoid and Park (1937). *Paramecium multimicronucleata* (a hideous hybrid and a false concord—*multimicronucleatum* being evidently intended) has already had quite a long run in American literature. America has also given us *Endamoeba bradypi*,[2] *Endolimax bradypi*, and *Giardia bradypi* (Hegner & Schumaker, 1928); and *Caryospora japonicum* (for *japonica*) has recently appeared in the Far East (Matubayasi, 1936). But the prize, in this class, must surely be awarded to *Boveria* "*teredinidi*"—a ciliate from the ship-worm, *Teredo* (gen. *teredinis*).[3] That so monstrous a specific name can nowadays pass muster as Latin—as it seemingly has done with numerous authors, editors, compositors, proof-readers, and reviewers, in civilized countries—is surely amazing.

Errors such as the foregoing are usually easy to correct (and their correction is authorized by the Rules of Nomenclature, as already pointed out): but there are other classes of new "Latin" names which may be more difficult to deal with. There are, for example, already many unacceptable specific names—and they are becoming increasingly frequent in protozoological writings—which are seemingly based upon a misapplication of Art. 14 (*c*) of the Code. According to this Rule, specific names dedicated to persons should be formed by the addition

[1] E.g. *Trichomitus termitidis* Kofoid & Swezy, 1919. This error was indicated, but not corrected, by Koidzumi (1921, p. 240).

[2] I am aware that Roman writers allowed *polypi* (as genitive of *polypus*); but this is irregular, and thus no good excuse for a modern author making *bradypi* as genitive of *βραδύπους*.

[3] See Nelson (1923) and Pickard (1927).

of *-i* to the proper name (*-ae* for a woman)—in imitation of the Latin genitives. (This gives us such curious but lawful forms as *möbiusi*, *mathisi*, *françai*, etc.) But there appears to be a widespread though quite unjustifiable belief in certain circles that this special rule can be applied indiscriminately to any noun whatsoever, in any language. Consequently, we now have—to take only a few recent instances—such astonishing specific names as the following: *Endamoeba dipodomysi* Hegner, 1926 (for an amoeba from *Dipodomys*, the kangaroo-rat); *Haemoproteus prognei*[1] Coatney & Roudabush, 1937 (for a blood-parasite of *Progne*, an American martin); *Hirmocystis parapeneopsisi* Setna & Bhatia, 1934 (for a gregarine from the Indian prawn *Parapeneopsis*); and even *Lernaeenicus seeri* Kirtisinghe, 1934 (for a copepod parasitic on an Indian seer-fish, *Cybium* sp.), and *Plasmodium heroni* Basu, 1938 (for a malarial parasite of an Indian heron, *Ardeola grayi*). How the last two specific names—and others like them—can be acceptably amended I cannot even suggest: I can only pray that they may ultimately vanish as invalid synonyms. If the prevailing fashion continues, however, we may shortly see such pleasing combinations as *Entamoeba monkeyi*, *Piroplasma dogi*, or *Trypanosoma guineapigi*.[2]

Attempts to correct seemingly false concords sometimes lead to peculiar results. An illustration of this is the strange name *Balantidium rhesum* proposed for a "new species" of this genus found in *Macacus rhesus* by Ghosh (1929). *Rhesus* is, of course, a proper name,[3] and stands as a noun in apposition to *Macacus* in the host's designation: there is no possible neuter adjective *rhesum*. As the species "*B. rhesum*" is almost certainly invalid, I suggest no emendation; but I note with regret that the specific name has been quoted already by Hegner (1934)[4] and other recent writers without comment or apparent qualm.

Among recent names which positively defy correction I may mention one which has just come to my notice. Bhatia and Setna (1938), after giving the

[1] This name is not merely an obvious atrocity in Latin and Greek: it also contravenes Art. 14 of the International Rules.

[2] Confirmation of this forecast comes unexpectedly from South Africa almost as I write: for a few days after I had written the above lines I received the *Onderstepoort J. vet. Sci.* **10**, no. 2, 1938 [issued in 1939] containing a paper (p. 253) on "Some Taenias from large wild carnivores" by R. J. Ortlepp. Among various new species therein proposed I note "*T. jakhalsi*" (*jakhals* being Dutch for a jackal), "*T. gonyamai*" (*gonyama*, it is stated, being Swazi for a lion), "*T. ingwei*" (*ingwe* being Zulu for a leopard)—and others of the same deplorable kind. In my view all such names should be rejected as violations of Art. 3 of the International Code: since it is obvious that the mere addition of *-i* to a Dutch, English, Zulu, or other non-Latin substantive neither makes it Latin nor "latinizes" it in any sense. (The name "*T. acinomyxi*"—proposed in the same paper for a parasite from *Acinonyx*, the cheetah—can be amended, however, though it exhibits manifest mistakes in spelling and declension.)

[3] The monkey was named *Simia Rhesus* by Audebert (1799; Fam. 2, Sect. 1, p. 5 [Pl. I]). He selected this classical proper name—instead of a descriptive adjective—on purpose, since it was one "*n'ayant aucune signification*".

[4] Who also himself proposed at the same time another "new species" *B. philippinensis* (presumably for *philippinense*, as *Balantidium* is neuter).

shocking generic name "*Contortiocorpa*"[1] to a gregarine from a polychaete worm (*Eunice*), propose to call another from the same host "*Deuteromera cleava* n.sp." The meaning of this specific designation is not immediately evident, though from the description of the gregarine it seems intended to denote "cleft". But there is no adjective "*cleava*", or any like it, in Latin: so the name, if accepted, can apparently be interpreted only as an arbitrary combination of letters.

Before I leave this subject I would take this opportunity of noting and deploring another modern usage which seems to me equally absurd: I mean the practice—now becoming fashionable in protozoology—of giving a host's generic name, in the nominative, as a specific name to its parasite. (The host's specific name is sometimes similarly transferred.) For example, a *Haemoproteus* from Californian quails of the genus *Lophortyx* is styled *H. lophortyx* (O'Roke, 1930):[2] two others from a humming-bird (*Archilochus*) and a grackle (*Quiscalus*) have been named *H. archilochus* and *H. quiscalus* respectively (Coatney & West, 1938): and a coccidian from a Japanese snake, *Natrix tigrina*, has lately been called *Koidzumiella*[3] *natrix* (Matubayasi, 1936). Many similar instances could easily be quoted from recent protozoological literature. Presumably such specific designations are valid under the International Code (Art. 14*b*), though they are glaring misnomers.[4] *Lophortyx* (from Gr. λόφος, hill and ὄρτυξ, quail) is a good enough generic name for a Valley Quail, but looks ridiculous when applied specifically to a protozoon in its blood. If this bad system of naming parasites is generally adopted we may expect to see a new malarial parasite of Man (*Homo sapiens* L.) some day labelled *Plasmodium homo* or *Plasmodium sapiens*.

We cannot fairly blame Oriental zoologists, who are very seldom educated in Greek and Latin, if they make occasional mistakes in the employment of these languages: we should rather sympathize with their difficulties. But it is our clear duty to correct their etymological errors—and also those of our Occidental (and European) brethren, with whom we can obviously have less sympathy because they have less excuse for their transgressions.

The Rules of Nomenclature were framed by reasonable men who had had the usual classical education and who naturally assumed that future generations of zoologists would possess at least an elementary knowledge of Greek and Latin. They also took it for granted that their successors would be equally reasonable, and that they would accept the ordinary conventions of language and printing. Accordingly, among the "Recommendations" of the old

[1] Introduced in 1935. The gregarine is described as having a twisted body, but this appears—from the description and figures—to be an artifact. There is good reason to hope, therefore, that the genus may be eliminated as a synonym, since it has no other obvious characters to distinguish it.

[2] The name was proposed originally in 1929, but I cite the chief publication.

[3] Later referred (Matubayasi, 1937) to *Tyzzeria* E. A. Allen.

[4] In the English version of Art. 14 there is also a glaring mistranslation of the French—so glaring that it has apparently escaped notice universally. The grammatical term *déclinaison* [=declension] is there mistranslated "declination"—a word which, of course, has several very different meanings in English.

Stricklandian Code[1]—the parent of our present Rules—the following (rather too hopeful) passage occurs: "In the construction of compound Latin words there are certain grammatical rules which have been known and acted on for two thousand years, and which a naturalist is bound to acquaint himself with before he tries his skill in coining zoological terms." How true—and how false!

Neither Strickland nor the other fathers of our present excellent Rules could have foreseen contemporary conditions: and consequently they made insufficient provision for the modern Indian or Japanese (or even American or South African) student—bound by no tradition, and with no classical background—who yet has to use Latin in naming his discoveries; or for any other zoologically competent workers who unfortunately have no knowledge of etymology or orthography, no literary sense, and no typographical taste. The Rules take much for granted which is not nowadays self-evident, apparently, in certain parts of the world. For instance, when they direct that generic names must be written with an initial capital, and specific with a small letter, they assume that other conventions will also be followed. "*Homo sapiens*" is orthographically correct, according to the Rules: but there is nothing in them to say that we may not write *HoMo sapienS*. We do not write like this because the Rules prohibit us, but because we adopt the ordinary linguistic conventions and possess a modicum of common sense and typographical good taste—and also because our printers, quite rightly, probably would not let us do this kind of thing, though they can hardly be expected to correct a word like "*Teranympha*" to *Teratonympha*. This is our job, as educated zoologists, and obviously we ought to do it ourselves.

New zoological names, as everyone will agree, should be neither proposed nor accepted without due deliberation; so I should like to terminate these remarks with another quotation from H. E. Strickland, whose wise words are always well worth remembering—"Our nomenclature presents but too many flaws and inelegancies already; and as the stern law of priority forbids their removal, it follows that they must remain as monuments of the bad taste or bad scholarship of their authors to the latest ages in which zoology shall be studied."

In conclusion, I will summarize what I have just written in order to emphasize the points I have tried to make. (1) The name "*Teranympha*" was a simple error in word-formation and orthography and should be corrected to *Teratonympha*. (2) The Rules of Nomenclature sanction this correction, which is conformable with Greek and Latin usage. (3) Other similar nomenclatural errors and solecisms should likewise be amended—whenever and wherever found. Outrageous and impossible "Latin" names should be cancelled. (4) Finally, I implore all future writers who are neither familiar with the Rules of Nomenclature, nor versed in Latin and Greek, to read the Rules (preferably in French) and consult a classical scholar before proposing any new

[1] Published 1842. I quote from P. L. Sclater's edition of 1878.

generic or specific names. Only by so doing can they avoid the introduction and perpetuation of barbarisms and absurdities like those which now increasingly corrupt and disfigure parasitology. With "Latin" zoological names —as with other human afflictions—prevention is better than cure.

REFERENCES

AUDEBERT, J. B. [1799]. *Histoire naturelle des Singes et des Makis.* Fol. Paris. (An VIII.)

BASU, B. C. (1938). Studies on a malarial infection in a paddy bird. *J. Malaria Inst. India*, **1**, 273.

BHATIA, B. L. & SETNA, S. B. (1938). On some gregarine parasites from certain polychete worms from the Andaman Islands. *Proc. Indian Acad. Sci.* (B), **8**, 231.

CLEVELAND, L. R. (1938). Morphology and mitosis of *Teranympha*. *Arch. Protistenk.* **91**, 442.

COATNEY, G. R. & ROUDABUSH, R. L. (1937). Some blood parasites from Nebraska birds. *American Midland Naturalist*, **18**, 1005.

COATNEY, G. R. & WEST, E. (1938). *Idem*, II. *Ibid.*, **19**, 601.

DOGIEL, V. (1917). *Cyclonympha strobila* n.gen. n.sp., a new representative of the family Trichonymphidae. [In Russian.] *J. Microbiol.* (Petrograd), **4**, 47.

GHOSH, E. (1929). A new species of Balantidium from the intestine of the Bengal monkey (*Macacus rhesus*). *J. Roy. Micr. Soc.* (Ser. iii) **49**, 14.

HEGNER, R. (1926). *Giardia beckeri* n.sp. from the ground squirrel and *Endamoeba dipodomysi* n.sp. from the kangaroo rat. *J. Parasit.* **12**, 203.

—— (1934). Specificity in the genus Balantidium [etc.]. *Amer. J. Hyg.* **19**, 38.

HEGNER, R. & SCHUMAKER, E. (1928). Some intestinal amoebae and flagellates from the chimpanzee, three-toed sloth, sheep and guinea-pig. *J. Parasit.* **15**, 31.

KIRTISINGHE, P. (1934). *Gloiopotes watsoni* n.sp. and *Lernaeenicus seeri* n.sp. [etc.]. *Parasitology*, **26**, 167.

KOFOID, C. A. & PARK, J. T. (1937). A new trematode, Paramphistomum castori sp.nov. [etc.]. *Univ. California Publ. Zool.* **41**, 419.

KOFOID, C. A. & SWEZY, O. (1919). On *Trichomitus termitidis* [etc.]. *Ibid.* **20**, 21.

KOIDZUMI, M. (1916). Studies on the trichonymphids parasitic in the termites of Japan. I. [In Japanese.] *Ann. Rept. Inst. Sci., Govt. Formosa*, **5**, 55. [28 pp. + 2 Pl.]

—— (1917). *Idem*, II. *Ibid.* **6**, 1. [84 pp. + 10 Pl.]

—— (1921). Studies on the intestinal protozoa found in the termites of Japan. *Parasitology*, **13**, 235.

MATUBAYASI, H. (1936). On a new coccidia [*sic*], *Koidzumiella natrix* n.g., n.sp. [etc.]. *Keiô-Igaku*, **16**, 1721. [In Japanese. English résumé, p. 1728.]

—— (1937). Studies on parasitic protozoa in Japan. II. Coccidia parasitic in snakes [etc.]. *Annot. Zool. Japon.* **16**, 255.

NELSON, T. C. (1923). On Boveria teredinidi, sp.nov. [etc.]. *Anat. Rec.* **26**, 356.

O'ROKE, E. C. (1930). The morphology, transmission and life-history of Haemoproteus lophortyx O'Roke [etc.]. *Univ. California Publ. Zool.* **36**, 1.

PICKARD, E. A. (1927). The neuromotor apparatus of Boveria teredinidi Nelson [etc.]. *Ibid.* **29**, 405.

SETNA, S. B. & BHATIA, B. L. (1934). On some gregarines from the prawn, *Parapeneopsis sculptilis* (Heller). *Parasitology*, **26**, 34.

[*MS. received for publication* 25. III. 1939.—Ed.]

THE CULICIDAE OF THE CAMBRIDGE DISTRICT

By T. T. MACAN, M.A., F.R.E.S.

From the Laboratory of the Freshwater Biological Association, Wray Castle, Ambleside, Westmorland

I. Introduction

The following notes are the results of week-end collecting during the academic years 1929 to 1933 and 1934–5. Records for June to September were only obtained during the last year. The object has been an investigation of the larval habitats of the different species of mosquito in an area which lies roughly within a ten-mile radius of Cambridge and includes three main formations, (*a*) chalk downs, (*b*) downs capped with boulder clay and (*c*) alluvial and fenland deposits.

II. The habitats

Aquatic habitats are not found on the chalk downs but occur abundantly both on the boulder clay and in the fens. There are relatively large bodies of water such as ornamental lakes, brick-pits, coprolite pits, horse-ponds, etc., but these are of no interest to the present paper as they rarely harbour any mosquitoes. These insects breed in smaller, shallower and often temporary bodies of water. On the impervious boulder clay such habitats are found almost wherever there is a hollow or slight excavation, particularly in the damp oak-ash woods of which there are a great many in the area. The fenland too provides many habitats of this nature. There are only a few small patches of natural fen with sedge and standing water, at least in the winter months, in the area; most of it is now cultivated and kept free of water by an intricate system of drains and ditches. Many of these have fallen into disuse and become stagnant and in them mosquitoes breed abundantly.

A type of breeding place which is quite independent of geological formation is the rain-filled tree-cavity or tree-hole. There are records from twelve different tree-holes occurring in four different species of tree, oak, elm, ash and chestnut. Most of the holes are formed where a branch has been blown or sawn off, but three of the most interesting, along the south edge of Toft Plantation adjoining Hardwick Wood, are formed by the rotting out of the boles of elm trees which have been cut down. The holes vary very much in size and may contain anything from a few hundred c.c. to ten or twenty litres of water.

III. Notes on the species

(1) *Aëdes cinereus* Meigen. I have not found this species in the cultivated fenland though it is fairly common in the natural fen of Wicken (Lewis, 1932) and Wood Walton.

(2) *Aë. geniculatus* Olivier. The larvae of this species have been found in eleven out of the twelve tree-holes. In each case it was the commonest species and usually it was present in large numbers. The most continuous series of observations were obtained from the Toft Plantation tree-holes and the records for two of them are reproduced in Table I. The localities are referred to by the initial letter of the parish in which they lie and a serial number, and the position of each one is shown on $\frac{6}{5}$ in. to the mile maps now in the archives of the Cambridge Natural History Society. C 3 was the largest tree-hole and held about 15 litres of water, C 22 was roughly half the size and the volume of C 2 was intermediate between the two. The course of events in C 2 was similar to that found in C 3 but it never contained either *Orthopodomyia pulchripalpis* or *Theobaldia morsitans*. The record of *T. morsitans* in a tree-hole is believed to be unique (Macan, 1930).

Table I. *Records from two tree-holes in Toft Plantation*

	C 3			C 22		
Date	*Aë. geniculatus*	*An. plumbeus*	*T. morsitans*	*Aë. geniculatus*	*An. plumbeus*	*O. pulchripalpis*
x. 29	Several hundred	About 6	About 6		No records	
25. iv. 30	Do.	Do.	Do.		Do.	
25. x. 30	Many Y	Many Y	—		Do.	
5. ii. 31	Y and O	Present	—		Do.	
18. iii. 31	M	—	—		Do.	
21. xi. 31	All instars	Y	—		Do.	
19. i. 32	All instars	—	—		Do.	
28. ii. 32	5 I, 25 II, 16 III, 17 IV	2	—		Do.	
11. v. 32	M	—	—		Do.	
16. x. 32	20	6	—	About 100 all instars	6	7
30. x. 32	Scarce	Scarce	—	130 all instars	30	8
12. iii. 33	27 I, 14 II, 1 III, 3 IV	—	—	130 I, 3 II, 5 III, 5 IV	7	—
5. x. 34	—	Dry	—	Dry. Scrapings of wood collected from inside of hole		
21. x. 34	—	—	—	Above scrapings put in water. Many *Aë. geniculatus* larvae emerged		
30. xii. 34	Many Y, about 100 O	—	—		Dry	
17. ii. 35	81 I, 173 M, 11 IV	12	—		Do.	
28. vii. 35	Mostly IV	—	—		Do.	

The Roman numerals refer to the instar, and when this was not precisely determined the following abbreviations are used: Y = young (approx. I), M = middle-aged (approx. II and III), O = old (approx. IV).

Among most species of *Aëdes* it is usual to find that in any one locality all the larvae are in approximately the same stage of development; for *Aë. geniculatus* on the other hand in any one tree-hole the larvae are found in all instars during many months of the year.

(3) *Aë. rusticus* Rossi is both the most numerous and the most widespread species in the area. It has been recorded from thirty-six different localities. Ten of these are woodland pools in which it is usually found with *Aë. cantans*, two are open pools on the boulder clay in which it was found with *Theobaldia*

morsitans and the rest were in the fenland where it was found alone in thirteen localities, with *T. morsitans* in six localities and with various associations of *T. morsitans*, *T. annulata*, *Aëdes cantans*, *Aë. annulipes* and *Anopheles claviger* in the remaining five localities. The breeding places of *Aëdes rusticus* are always small and shallow. In the smallest and shallowest which dry out in summer it is found alone or, if the pools be within a wood, with *Aë. cantans*. In larger deeper pools where the water shrinks considerably during the summer, but does not disappear completely, except perhaps for a short period, *Aë. rusticus* is usually found with *Theobaldia morsitans*, *T. annulata* and other species. The breeding places usually have little or no vegetation.

The adults appear in May and early June and lay their eggs on damp ground where they remain dormant until covered by water. The date on which a pool fills up will differ widely from year to year and it is of course widely different for different pools in the same year, but apparently the eggs hatch at whatever time of year water first covers them, for I have found first instar larvae in all months from October to March inclusive. Once the eggs have hatched larval development proceeds regardless of the time of year, till the fourth instar is reached, for I have recorded this stage in all months from December to June inclusive. Apparently such larvae remain in the fourth instar until spring for my earliest pupal record is 27 April. In a dry spring the pools often dry up before the larvae have pupated, while heavy rain sometimes washes larvae breeding in a stagnant fenland ditch into a larger flowing ditch where their chances of survival are small.

(4) *Aëdes annulipes* Meigen is found occasionally in the fenland.

(5) *Aë. cantans* Meigen has been found in vast numbers in temporary pools in Swanseley, Great Covens, Eversden, North and Kingston Woods and probably occurs in most of the woods in Cambridgeshire. In all the woods *Aë. rusticus* was also present though in smaller numbers. A few specimens of *Aë. cantans* have been found breeding in the fenland.

(6) *Theobaldia annulata* Schrank is a well-known "domestic" mosquito which breeds in water-butts, garden tanks, etc. Larvae appear in "country" habitats in large numbers in late June and July and, though I have only one summer's records, these suggest that it breeds abundantly in all the different types of locality, particularly in the fens. Three localities were ditches where *Aëdes rusticus* had bred earlier in the year. By July they were nearly dry and water was confined to a few small pools. In these pools larvae of *Theobaldia annulata* were abundant. The species has a well-known predilection for foul habitats, as is shown by its occurrence together with *Culex pipiens* in the effluent tanks of the bone-meal factory at Fulbourn.

A few larvae, and occasionally even pupae, are to be found in the fens throughout the winter though this species hibernates principally as an adult.

(7) *Theobaldia morsitans* Theobald. Mosquitoes were collected in the Cambridge District by the Rev. L. Jenyns in and around the year 1840. Among specimens labelled *T. morsitans* in his collection Marshall in 1932 found

examples of his newly described species *T. litorea*. In response to a request I sent him in 1933 a collection of larvae of what I had been wont to call *T. morsitans*. The larvae were bred out at the British Mosquito Control Institute and proved to be a mixed population of which eighteen were *T. litorea* and only three were *T. morsitans*. Clearly *T. litorea* is a fenland species and the following notes on *T. morsitans*, made prior to 1933, must in many cases refer to *T. litorea* also, and the same is likely to be true of Lewis's (1932) Wicken Fen records.

The species is widespread in the district but it does not occur in large numbers. Shute records taking a thousand larvae during the course of an hour's collecting in November; in the Cambridge District twenty-five in an hour would be a good catch.. It has been found in one tree-hole, four woodland pools or ditches, five open pools on the boulder clay and in fifteen localities in the fens. In six localities it occurred alone, in ten it was found with *Aëdes rusticus*, in two with *Anopheles claviger*, in two with *Theobaldia annulata*, in three with both species together and in one locality it was found with *Anopheles maculipennis*. *Theobaldia morsitans* larvae are found in temporary collections of water which, however, retain their water for a longer period than the average breeding place of *Aëdes rusticus* and often have a small central pool which rarely dries up. The breeding places of this species also tend to be more overgrown with vegetation, particularly with *Phragmites*, than those of *Aëdes rusticus* but whether this is due to a preference for shade on the part of the ovipositing female, or merely to the damper conditions, only experimental work can show.

I have no records about the emergence or oviposition of this species, but have found it four times in the fourth and once in early instars in October and in the fourth instar in all subsequent months to May inclusive. Wesenberg-Lund (1921) and Marshall (1938) state that the eggs are laid in July and August.

(8) *Theobaldia litorea* Shute. See *T. morsitans*.

(9) *Culex pipiens* Linnaeus, like *Theobaldia annulata*, appears in country districts in large numbers from about midsummer onwards but, unlike it, disappears in the autumn and passes the winter exclusively in the adult stage.

(10) *Taeniorhynchus richiardii* Ficalbi. The unusual habit of tapping plant roots for respiratory purposes possessed by the larva of this species renders it difficult to find and I have only one record—from a fen ditch with *Phragmites*—though Lewis (1932) records it as "an abundant biter in July" in Wicken Fen.

(11) *Orthopodomyia pulchripalpis* Rondani. In 1927 Keilin described the fauna of a horse-chestnut tree in which this species occurred. In 1932 Macan & Tutin described another locality close by, also in a chestnut tree, and later in the same year a third locality (C 22) was discovered in Toft Plantation. In the following spring *O. pulchripalpis* was not found in this last tree-hole though the other two species, *Aëdes geniculatus* and *Anopheles plumbeus*, were as common as before (see Table I). On all subsequent visits the tree-hole has been dry. *Orthopodomyia pulchripalpis* has not been found in any of the nine other tree-holes known in the district.

All three Toft Plantation tree-holes (C 2, C 3, C 22) are formed in the same way in the sort of same tree and the situation of all three is identical (p. 263). All three harbour *Aëdes geniculatus* and *Anopheles plumbeus* but it is only in C 22 that *Orthopodomyia pulchripalpis* has been found. This hole is smaller than the other two, has a narrower opening and appears to be less liable to flooding, for on 16 October 1932 and again on 30 October 1932 after heavy rain both C 2 and C 3 appeared to have been overflowing. The water was of an unusually light colour and larvae were very scarce. Conditions in C 22 were unchanged. Possibly as a result of this tendency C 22 has normally a darker colour than the other two and also a higher *p*H (C 22 9·4, C 2 8·2, C 3 8·2). The original tree-hole described by Keilin had a *p*H of 9·0 and the one near it described by Macan & Tutin a *p*H of 8·5.

(12) *Anopheles maculipennis* Meigen is found in open pools during the summer months. Lewis (Cambridge Natural History Society fauna slips) records it from backwaters of the river Cam.

(13) *A. claviger* Meigen has been recorded from thirteen localities of which ten are in the fens and are pools or ditches with a growth of *Phragmites*. It has been found alone once, with *Theobaldia annulata* once, with *Aëdes rusticus* once, with *Aë. rusticus* and *Theobaldia morsitans* once, with *T. morsitans* and *T. annulata* three times, and with *Aëdes rusticus* and *Theobaldia annulata* four times.

(14) *Anopheles plumbeus* Stephens has been recorded from nine out of the twelve tree-holes visited. It will be seen from Table I that its numbers were commonly much less than those of *Aëdes geniculatus* in the Toft Plantation tree-holes, an observation which was found to hold good for all the rest except one near Dullingham where a count showed thirty-five *Anopheles plumbeus* to fifty-one *Aëdes geniculatus* and in the second tree-hole described by Macan & Tutin (1932) where it was the only species present. This was one of the smallest holes; in two other very small ones *Aë. geniculatus* was the only inhabitant.

IV. COMPARISON WITH OTHER DISTRICTS

Theobaldia litorea and *Orthopodomyia pulchripalpis* are listed by Marshall (1938, p. 28) among the rarer British species. The rarity of *Theobaldia litorea* is possibly due to the fact that it has only recently been separated from *T. morsitans*, a species which it resembles very closely; the rarity of *Orthopodomyia pulchripalpis* is undoubtedly genuine. Apart from these two the mosquitoes found in the Cambridge District belong to common and widely distributed species. It is, however, interesting to note that in this district, where there is an abundance of lime in the soil, *Aëdes rusticus* is the commonest species and *Aë. punctor*, typical of sandy and peaty "acid" stretches of country, has not been recorded.

An interesting comparison may be made between the mosquito fauna of cultivated fenland and that of "natural" fenland such as Wicken Fen. The

water level in Wicken Fen is controlled with a view to keeping the soil in a permanently waterlogged condition, so that, except in the middle of a dry summer, there is always standing water in the sedge. In the cultivated fenland extensive drainage schemes are always in action to keep the soil free of water. Lewis (1932) has investigated the mosquitoes of Wicken Fen. He writes: "*Culicella morsitans* is probably the commonest mosquito of the Fen. Its larvae are abundant in shallow water in the sedge...." *Aëdes cinereus* is also recorded as fairly common. *Aë. rusticus* is listed among the species which are least numerous in the fen. His observations on the remaining species suggest that their frequency in Wicken and in cultivated fen is similar. It appears, therefore, that the reclaiming of the fenland and the disappearance of the sedge leads to a considerable reduction in the numbers of *Aë. cinereus* and a less marked reduction in the numbers of *Theobaldia morsitans*, while the corresponding appearance of ditches leads, when these are allowed to get choked up and stagnant, to a great increase in the numbers of *Aëdes rusticus*.

V. Notes on ecology

The larvae of some species, the most striking example being the tree-hole breeders, are only found within a narrow range of habitats. The remaining species may be arranged in a series in which the range of habitats becomes increasingly wider. Thus, of the mosquitoes discussed above, *Aëdes cantans* is seldom found outside woods, *Aë. rusticus* may perhaps be classed as "calciphilous", and *Theobaldia morsitans*, *T. annulata* and *Culex pipiens* are found breeding in an extremely wide range of habitats. Among these three, however, "preferences" may be detected, thus *Theobaldia morsitans* is usually found where there is emergent vegetation (see, however p. 266), and *T. annulata* and *Culex pipiens* are the only species found breeding in water-butts and highly polluted habitats. The occurrence of *Anopheles maculipennis* in open, and *An. claviger* in shaded, habitats may also be mentioned.

Aëdes rusticus and *Theobaldia morsitans* oviposit on damp ground; *T. annulata*, *Culex pipiens*, *Anopheles maculipennis* and *An. claviger* oviposit on the surface of standing water. *Aëdes rusticus* oviposits in May or June, the rest a month or two later. Small shallow collections of water which dry completely early in the summer will provide damp conditions for *Aë. rusticus* but will be quite dry when *Theobaldia morsitans* is ready to lay its eggs. Small but deeper collections of water which dry completely will be full when *Aëdes rusticus* is ovipositing but will provide a suitably damp locality for the eggs of *Theobaldia morsitans* later in the year. A large shallow collection of water in which the water shrinks rapidly throughout the summer will provide suitable damp conditions at the time of oviposition of both species, and if there is a deeper central pool where water stands all the year round suitable conditions for the oviposition of *T. annulata*, *Culex pipiens* and the two species of *Anopheles* will also be provided.

There should, therefore, be a close correlation between the area and depth of a locality and its mosquito population. Such a correlation has actually been found in the field, and it appears that, in the fenland, though factors such as shading and degree of pollution may play a part in the ecology of the mosquitoes found, the most important factor is the different oviposition habits of the various species.

SUMMARY

1. The mosquito fauna of the Cambridge district, which includes a large stretch of cultivated fenland, is compared with that of the "natural" fen at Wicken and also with that of other districts.

2. The ecology of the various species is discussed and the conclusion is reached that the main factor determining why different species are found in different types of habitat is the time of year at which the female lays her eggs, and whether she lays them on damp ground or standing water.

REFERENCES

KEILIN, D. (1927). Fauna of a horse-chestnut tree (*Aesculus hippocastanum*), dipterous larvae and their parasites. *Parasitology*, **19**, 368–74.

—— (1932). On the water reservoir of a horse-chestnut tree. *Parasitology*, **24**, 280–2.

LEWIS, D. J. (1932). The mosquitoes of Wicken Fen. In *The Natural History of Wicken Fen, Cambridge*, (6), 548–59.

MACAN, T. T. (1930). *Culicella morsitans* in a tree-hole. *Entomologist*, **63**, 164.

—— (1933). An inland record for *Theobaldia* (*Culicella*) *litorea*. *Entomologist*, **66**, 261.

MACAN, T. T. & TUTIN, T. G. (1932). A note on rot-holes in horse-chestnut trees. *Parasitology*, **24**, 283.

MARSHALL, J. F. (1938). *The British Mosquitoes*. British Museum (Natural History).

WESENBERG-LUND, C. (1921). Contributions to the biology of the Danish Culicidae. *D. Kgl. Danske Vidensk. Selsk. Skrifter, naturv. og mathematisk*, Copenhagen, **7**, 1–210.

(*MS. received for publication* 4. III. 1939.—Ed.)